INVESTIGATION OF FRUGIVORY IN NORTH AMERICAN MIGRATORY SONGBIRDS USING STABLE CARBON AND NITROGEN ISOTOPE ANALYSES

A Thesis

Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Biology, University of Saskatchewan, Saskatoon.

By

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ABSTRACT

Several species of North American migratory songbirds reportedly experience seasonal diet shifts involving a shift from an insect diet during the breeding season to one incorporating fruits during migration and non-breeding periods but the extent to which dietary plasticity occurs in migratory songbirds is poorly quantified. Thus, I used stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses to evaluate the timing and extent of frugivory throughout the annual life cycle of 16 species of migratory songbirds, representing wide ranges in body size and reported diets. Birds were sampled during spring and fall migration at the Delta Marsh Bird Observatory in 2003. To investigate dietary patterns, I sampled multiple tissues (muscle, liver, whole blood, claws, bone collagen, feathers) as these represent different periods of diet integration due to varying elemental turnover rates.

Assuming that relatively low $\delta^{15}N$ values represent a fruit diet and relatively high $\delta^{15}N$ values represent an insect diet, I expected tissues representing fall migration (liver, blood, and muscle from fall-captured birds) and winter (greater coverts and claws from spring-captured birds) to have lower $\delta^{15}N$ values than tissues representing spring migration (liver, blood, and muscle from spring-captured birds) and summer (tail feathers and claws from fall-captured birds) when fruits are presumed to be less common in songbird diets. Based on blood and claw $\delta^{15}N$ values, there was no isotopic segregation of species I classified *a priori* as insectivores or omnivores. For most species, tissue $\delta^{15}N$ values showed either no seasonal change or a shift *opposite* to my prediction (e.g., $\delta^{15}N$ values higher in fall birds compared to spring birds). Boreal fruit $\delta^{15}N$ values were lower than those for insects; however, $\delta^{15}N$ values of agricultural fruits overlapped both boreal fruit and insect values suggesting that food web baselines did not conform to a simple (single) linear trophic-enrichment model. In Yellow-rumped Warblers (*Dendroica coronata*), within-tissue seasonal comparisons for liver, muscle and blood indicated a fruit diet during fall and winter and an insect diet during spring and summer; claws and feathers of birds captured in spring (representing winter diet) had unexpectedly high $\delta^{15}N$ values. Diet-tissue isotopic discrimination factors associated with both a fruit diet and insect diet were taken from the literature and used to

correct stable isotope values of tissues to putative diet because, currently, little is known about the nature of factors influencing discrimination factors to be used in simple linear dietary mixing models. There were differences in tissue $\delta^{13}C$ and $\delta^{15}N$ values depending on which discrimination factor was used. Based on mixing model results for tissue $\delta^{15}N$ values, a higher proportion of insects vs. fruits was detected in the diet of Yellow-rumped Warblers for all tissues except muscle and claws.

My interpretations are contingent on the fact that the available natural history information, on which guild classifications were based, was correct and that elemental turnover rates and discrimination factors used were accurate. However, much uncertainty remains about the appropriate diet-tissue isotopic discrimination factors corresponding to fruit and insect diets. Due to extensive natural variability of stable nitrogen isotope values in food sources, possible anthropogenic influences and a lack of knowledge of the metabolic processes that can potentially affect stable isotope values, I caution against using stable isotope analysis alone to track frugivory in temperate North American migratory songbirds. Future research should focus on captive studies aimed at determining and validating discrimination factors of various tissues, particularly claws and feathers, for birds feeding on varying proportions of fruits and insects. Additionally, more information on the dietary habits of these migratory songbirds is needed, as previous estimates of insectivory and frugivory in songbirds may not be accurate.

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DEDICATION

À ma famille et à grand-maman Maltais

"*Votre amour, soutien et encouragement au cours de cette aventure m'a été inestimable et pour cela, je vous remercie de tout mon cœur.*"

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CHAPTER 1: GENERAL INTRODUCTION

1.1. INTRODUCTION

Each year in North America, over two thirds of all breeding bird species migrate from the northern Boreal forest of Canada and Alaska to winter in the southern United States and Neotropics (Moore 2000). This journey requires migratory landbirds to have considerable ecological and physiological plasticity (Gwinner 1990) since they encounter a variety of conditions *en route* and on breeding and wintering habitats where they must adapt to changes in food, competitors and predators (Petit 2000). The most important limitation during a migratory bird's annual cycle is the challenge of acquiring enough food to meet its energetic requirements (Moore 2000). As such, a common adaptation to these changing environmental pressures is dietary plasticity, often seen as a shift from an insect diet during breeding to a fruit-dominated diet during fall migration and winter (Greenberg 1981, White and Stiles 1990, Parrish 1997, Borgmann et al. 2004, Herrera et al. 2005). This behaviour has been documented to varying degrees in several species of migratory songbirds (Parrish 1997, 2000), but mechanisms driving seasonal diet shifts remain unclear. Frugivory may reflect a change in food availability (Bairlein 1990, Berthold 2001) or be associated with changes in food preference and/or nutritional requirements (Bairlein 1990, Bosque and Pacheco 2000, Gannes 2001). The choice of fruit is often species-specific and not always related to local fruit abundance (Greenberg 1981, Malmborg and Willson 1988, Kwit et al. 2004a) and so fruits represent a food source that may help with fat deposition necessary for migrants (Moermond and Denslow 1985, Bairlein 1996).

The degree of dietary plasticity in migratory birds deserves study as such an understanding will provide insights into the evolution of strategies used by avian migrants in North America and elsewhere. Since the choice of food resources is inextricably linked to habitats (Moore 2000), in-depth knowledge of the extent of frugivory in North American migratory songbirds is also crucial to understanding

habitat and dietary requirements necessary for making conservation and management decisions. However, to date, few studies have been conducted in temperate zones to evaluate diet variation in omnivorous migratory songbirds (Parrish 1997, Parrish 2000, Suthers et al. 2000).

Previously, frugivory in songbirds was assessed using fecal sample and stomach content analyses (e.g., Johnson et al. 1985, Blake and Loiselle 1992, Parrish 1997, Prather 2000). Although these conventional techniques are effective and often non-destructive, there are major disadvantages such as a potentially biased representation of ingested food items due to differential rates of passage and processing of food types in the gut. Individuals with empty stomachs provide no information on types of prey eaten (Rosenberg and Cooper 1990) which preclude the collection of accurate data.

Stable isotope analysis has become an increasingly important tool for avian ecologists. The application of measurements of naturally occurring stable isotopes of various elements (e.g. H, C, N, O, S) to dietary studies is based on the fact that a bird's tissues will reflect the isotopic signatures of local food webs, which in turn vary according to several biogeochemical processes (Hobson 1999a). Stable isotopes are measured as ratios of heavy (e.g., ^{13}C or ^{15}N) to light (e.g., ^{12}C or ^{14}N) isotopes relative to those in international standards. All values are reported in δ (delta) notation according to the following equation (Ehleringer and Rundel 1989):

$$\delta x = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1.1)$$

where R_{sample} is the isotope ratio of the sample and $R_{standard}$ is the isotope ratio of the standard. This technique has been successfully used to infer feeding locations (Marra et al. 1998; Wassenaar and Hobson 2000); examine trophic relationships (Hobson et al. 1994, Herrera et al. 2003); trace nutrients to reproduction (Hobson et al. 2000a); reconstruct diet, and determine the relative contribution of isotopically distinct food sources (Wolf and Martinez del Rio 2000, Herrera et al. 2005).

Stable-carbon and nitrogen isotopes are particularly useful in the study of avian diets. In terrestrial environments, carbon enters the food web as atmospheric CO_2 with a $\delta^{13}C$ value close to -8 ‰ (Farhquar et al. 1989). This $\delta^{13}C$ value changes according to the distinct photosynthetic pathways of C_3, C_4, and CAM plants which have different methods of carbon fixation (Lajtha and Marshall 1994). C_3 plants utilize the RuBisco

photosynthetic pathway whereby atmospheric CO_2 enters the Calvin cycle and binds to a sugar molecule via the enzyme rubisco (ribulose bisphosphate carboxylase). C_3 plants, which are common in temperate areas, discriminate against ^{13}C in favour of ^{12}C and therefore have ^{13}C-depleted tissues and a mean $\delta^{13}C$ value close to -27 ‰ (Tieszen and Boutton 1989). The Hatch-Slack photosynthetic pathway occurs in C_4 plants which are more typical of xeric environments. In this process, atmospheric CO_2 is bound to an organic compound by a different enzyme, phosphoenolpyruvate (PEP) carboxylase, which has a higher affinity for CO_2 than Rubisco, which is then released into the Calvin cycle. The C_4 photosynthetic pathway minimizes photorespiration and enhances sugar production. These plants have ^{13}C-enriched tissues with $\delta^{13}C$ values between -9 and -14 ‰ (Ehleringer and Rundel 1989). Crassulacean acid metabolism (CAM) plants have a photosynthetic pathway similar to C_4 plants with the major difference being that in C_4 plants, the initial steps of carbon fixation are separated structurally from the Calvin cycle whereas in CAM plants, the two steps occur at separate times (carbon fixation at night and the Calvin cycle during the day). As such, CAM and C_4 plants cannot be distinguished using stable carbon isotopes (Lajtha and Marshall 1994) but can be differentiated using deuterium measurements (Smith and Ziegler 1990). Also, stable isotope analysis can distinguish between C_3 plants that have different water-use efficiency (WUE) which is defined as the ratio of net photosynthesis to transpiration (Lajtha and Marshall 1994). C_3 plants in moist environments have stomatas which are more open and will discriminate to a greater extent against ^{13}C when taking in CO_2 for photosynthesis. On the other hand, C_3 plants found in xeric habitats will reduce the size of their stomatal opening to reduce water loss, consequently reducing transpiration and increasing WUE, and so are less able to discriminate against ^{13}C and having tissues more ^{13}C enriched. As such, C_3 plants in moist habitats have lower $\delta^{13}C$ values than C_3 plants in dry habitats (Lajtha and Marshall 1994). Consumers will have tissue $\delta^{13}C$ values similar to the $\delta^{13}C$ value of their diet, within about 1 ‰ (DeNiro and Epstein 1978). The isotopic signature of carbon varies little throughout food webs and so $\delta^{13}C$ analysis can be used to trace origin of nutrients within individuals based on their choice of plant-based food webs (Hobson 1999a) and determine whether feeding occurred in xeric or moist C_3 habitats (Marra et al. 1998, Chamberlain et al. 2000).

Stable nitrogen isotope measurements play an important role in describing trophic levels. In terrestrial ecosystems, $\delta^{15}N$ values vary in soils and plant tissues. The discrimination against ^{15}N occurs in nitrogen cycles, depleting vegetation and litter of ^{15}N and enriching humus in ^{15}N. Plant tissues generally have $\delta^{15}N$ values ranging from -5 to +2 ‰ (Nadelhoffer and Fry 1994). Dietary ^{15}N will be preferentially incorporated into animal tissues due to processes associated with differential excretion of ^{14}N (Hobson 1999c). Free amino acids originate, in part, from the digestion of dietary proteins. These amino acids constitute an important source of nitrogen in most animals (Kelly 2000). When these amino acids enter the bloodstream, they are absorbed by other tissues, primarily the liver or muscle. Here, amino acids are converted to ammonia and carbohydrate through the process of deamination or transamination. Next, ammonia is converted to urea and then to uric acid, in the case of birds, and eventually excreted (Voet et al. 1999; Kelly 2000). There is evidence that ^{14}N is preferentially excreted over ^{15}N during this last step (Minagawa and Wada 1984) and that the discrimination which occurs during production of nitrogenous wastes is greater than nitrogen absorption from the diet (Kelly 2000). Therefore, animal tissues are generally enriched in ^{15}N compared to their diet (Kelly 2000) and consumers become ^{15}N enriched with increasing trophic level. Enrichment in ^{15}N ranges from about 2.5 to 5 ‰ for each increase in trophic level (Peterson and Fry 1987, Kelly 2000), with an average of about 3.4 ‰ (Kelly 2000, Post 2002). Therefore, diet shifts (e.g., herbivory vs. insectivory) can potentially be identified by quantifying $\delta^{15}N$ variations in tissues of consumers (e.g., Herrera et al. 2003, Herrera et al. 2005).

The application of stable isotope analysis to studying avian diets depends on knowledge of two important factors: 1) turnover rates of isotopes in tissues of wild birds and 2) diet-tissue isotopic discrimination factors, the amount of change that occurs in dietary stable isotope values when isotopes are incorporated into tissues. Because stable isotope ratios of elements are measured in tissues of consumers, the use of this technique augments traditional methods by providing information on assimilated diet versus recently ingested foods over a period of days to months depending on the choice of tissues. Different tissues represent assimilated diet over different timeframes due to

varying elemental turnover rates (Hobson and Clark 1992a). Therefore, by using multiple tissues, an individual's diet can be reconstructed for various time periods.

Turnover rate refers to the rate at which a given tissue incorporates the isotopic signal of a new diet (Hobson and Clark 1992a). Controlled laboratory studies have been conducted to determine turnover rates of various avian tissues by switching captive birds from one isotopically distinct diet to another and measuring the isotopic rate of change in each tissue (Hobson and Clark 1992a, 1992b, 1993, Haramis et al. 2001, Bearhop et al. 2002, Pearson et al. 2003, Hobson and Bairlein 2003, Evans-Ogden et al. 2004, Podlesak et al. 2005). Captive birds have included the American Crow (*Corvus brachyrhynchos*), Japanese Quail (*Coturnix japonica*), Ring-billed Gull (*Larus delawarensis*) (Hobson and Clark 1992a, 1992b, 1993), Canvasback (*Aythya valisineria*) (Haramis et al. 2001), Great Skua (*Catharacta skua*) (Bearhop et al. 2002), Dunlin (*Calidris alpina*) (Evans-Ogden et al. 2004), Garden Warbler (*Sylvia borin*) (Hobson and Bairlein 2003), and Yellow-rumped Warbler (*Dendroica coronata*) (Pearson et al. 2003). Tissues with fast turnover rates, such as liver and blood plasma, reflect diet over a short period of 3 to 6 days while tissues with slower turnover rates, such as whole blood and muscle, reflect diet over a longer period of 2 to 3 weeks (Hobson and Clark 1992a, Bearhop et al. 2002, Pearson et al. 2003, Evans-Ogden et al. 2004). A diet integration period is considered to be the time for a tissue to largely incorporate a new diet's isotopic signature (arbitrarily, 2 to 3 half-lives of the exponential turnover pattern or 75-87.5% of the tissue element representing new diet). Turnover rates derived from captive studies are used as approximations of turnover rates in wild birds. There is a linear relationship between the elemental turnover rate of a tissue and its degree of metabolic activity (Tieszen et al. 1983). Wild birds may have higher metabolic rates than captive birds and, consequently, they may have higher elemental turnover rates (Hobson and Clark 1992a, but see Hobson and Yohannes 2007). Similarly, smaller birds may have higher tissue turnover rates compared to larger birds because of their higher metabolic rates (Tieszen et al. 1983, Pearson et al. 2003). Therefore, it is important to choose turnover rates from a species which closely resembles the target study species.

To derive quantitative estimates of the relative contribution of isotopically distinct dietary components using isotope mixing models (Phillips 2001), precise estimates of diet-tissue discrimination factors are required. These values have been determined during controlled laboratory studies, as described above. Although initial studies solely focused on providing discrimination factors for one isotope (e.g., Hobson and Clark 1992b, Podlesak et al. 2005), increasingly, studies have calculated discrimination factors for both carbon and nitrogen isotopes in the same tissue (Bearhop et al. 2002, Hobson and Bairlein 2003, Pearson et al. 2003, Podlesak and McWilliams 2006). Discrimination factors depend on diet and tissue. The majority of studies have determined discrimination factors for birds fed either a plant-based or animal-based diet. However, Pearson et al. (2003) fed Yellow-rumped Warblers an omnivorous diet and found that birds fed a fruit-dominated diet had lower $\delta^{15}N$ discrimination factors than birds fed an insect-dominated diet both within and between tissues. This study was contradicted by Robbins et al. (2005) who found no relationship between $\delta^{15}N$ discrimination factors and dietary nitrogen content. For animals feeding on food sources which differ in elemental concentrations, it has been suggested that a concentration-dependent mixing model should be used (Phillips and Koch 2002, Pearson et al. 2003). Also, differential metabolic routing of macronutrients from diet to tissue is another factor which may influence diet-tissue discrimination factors (Haramis et al. 2001, Bearhop et al. 2002, Evans-Ogden et al. 2004, Podlesak and McWilliams 2006).

1.2. STUDY AREA

Fieldwork was conducted during spring and fall migration 2003 at the Delta Marsh Bird Observatory (DMBO), located in the dune-ridge forest of Delta Marsh, MB, Canada (Fig. 1.1; 98°23'W, 50°11'N). Delta Marsh is one of largest lacustrine marshes in North America (MacKenzie 1982). The narrow ridge forest which abuts the south end of Lake Manitoba is a primary stopover site for migratory songbirds *en route* to and from their breeding and wintering grounds. Because large numbers of migrating songbirds concentrate in this area, the DMBO captures an average of 7,500 birds per year (den Haan, Delta Marsh Bird Observatory director, unpublished data) and provides

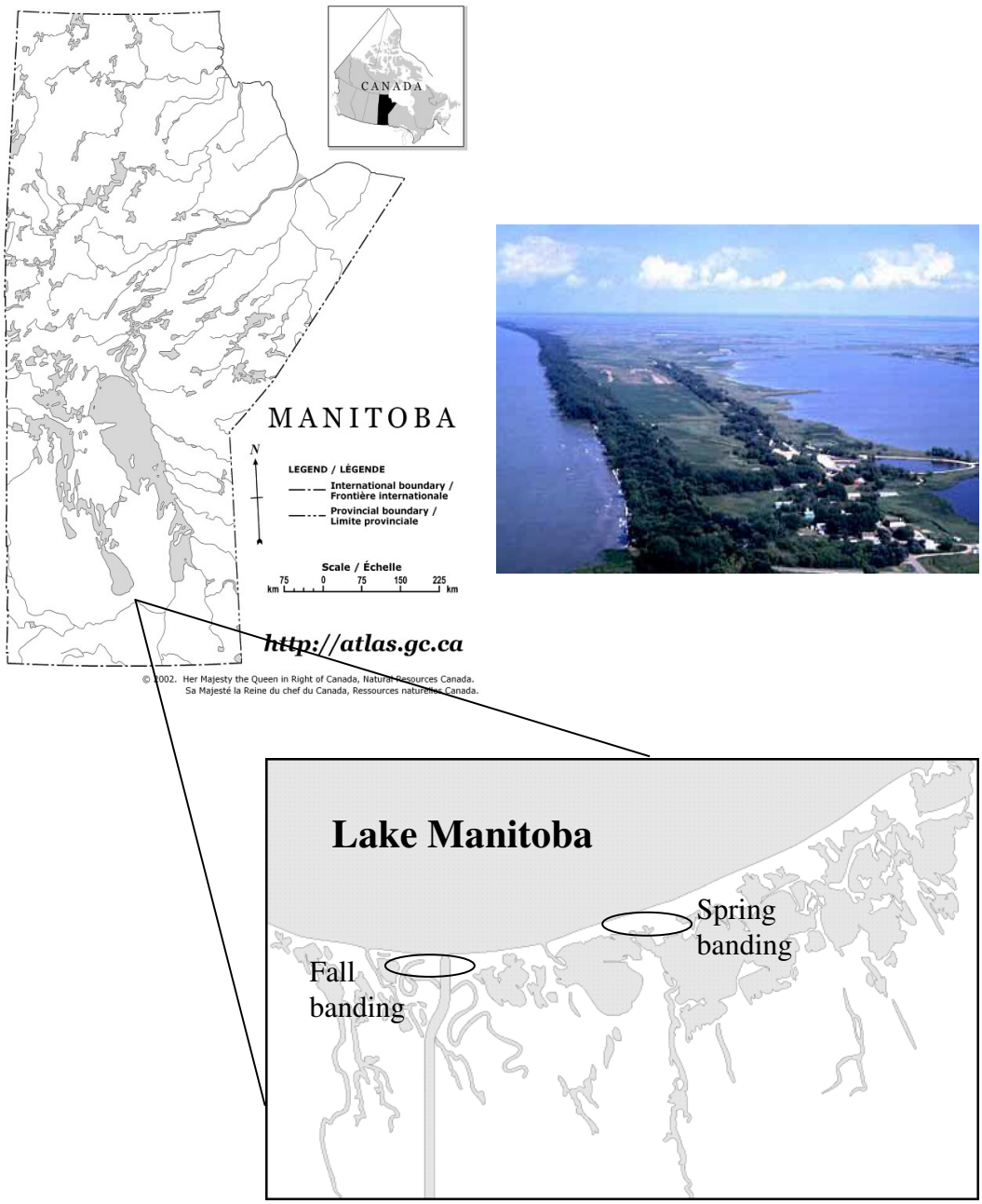


Figure 1.1 Aerial view of the dune-ridge forest at Delta Marsh, MB (top right). Locations of the spring and fall banding sites of the Delta Marsh Birds Observatory where migratory songbirds were captured in 2003 (bottom).

access to a variety of species. This study area is located in the aspen parkland region approximately 170 km south of the boreal forest.

1.3. STUDY SPECIES

My research focused on sixteen species of migratory songbirds with an emphasis on Yellow-rumped Warblers. These species were chosen to represent a range of dietary habits incorporating varying amounts of fruits and insects. Knowledge of each species' diet was based largely on corresponding accounts in Birds of North America (Poole 2005) and quantitative studies (Parrish 1997). Species were subsequently classified as insectivore or omnivore (i.e., fruit and insect diet) guilds. I also considered abundance of each species migrating through the DMBO based on banding data, 1992-2002 (den Haan, unpublished data), when choosing study species to ensure adequate sample sizes.

1.3.1. Yellow-rumped Warbler

The Yellow-rumped Warbler is one of the most common warblers in North America and consists of two distinct groups formerly considered separate species: Myrtle Warbler (*coronata* group) of northern and eastern North America and Audubon's Warbler (*auduboni* group) of western North America and Mexico (Hunt and Flaspohler 1998). Only Myrtle Warblers were present at my study site. This species has a broad geographic range in North America with its breeding range extending from Alaska east to the northeastern United States and eastern Canada, and south to Guatemala, and wintering in the southern United States, Mexico, and western Caribbean (Hunt and Flaspohler 1998). Yellow-rumped warblers are usually one of the first migrants in spring (arriving in April) and one of the last migrants in fall (departing in October) (Hunt and Flaspohler 1998). During breeding season, this species occurs primarily in mature coniferous and mixed coniferous-deciduous habitats and feeds on insects and other small invertebrates. Yellow-rumped warblers occupy a variety of habitats during non-breeding periods and expand their diet to include a large proportion of fruits during fall and winter, particularly northern bayberry (*Myrica pensylvanica*) and wax myrtle (*Myrica cerifera*) (Hausman 1927, Martin et al. 1951, Bent 1953, Borgmann et al. 2004, Kwit et al. 2004a). This species also eats berries from juniper (*Juniperus* spp.), Virginia

creeper (*Parthenocissus* spp.), viburnum (*Viburnum* spp.), honeysuckle (*Lonicera* spp.), mountain ash (*Sorbus* spp.), poison ivy (*Toxicodendron radicans*), poison oak (*T. toxicodendron*), greenbrier (*Smilax* spp.), and dogwood (*Cornus* spp.) (Hunt and Flaspohler 1998). During breeding, the proportion of insects in the diet of Yellow-rumped Warblers appears to be greater for birds in the west than their eastern counterpart (Forbush 1929 in Hunt and Flaspohler 1998). Few quantitative studies have examined their diet during migration and winter. Yarborough and Johnston (1965) documented 78% plant material (mostly bayberry) for Yellow-rumped Warblers along the coast of North Carolina and 51% plant material (mostly berries of red cedar) for birds inland with a shift to 100% insect material during the premigratory period. Parrish (1997) reported 82% fruit material in fecal samples of Yellow-rumped Warblers on fall migration along the eastern coast of Rhode Island.

1.3.2. American Redstart (*Setophaga ruticilla*)

The American Redstart has a broad geographic range breeding across southern Canada north to Yukon and most of eastern and northern United States. It winters in Mexico, Central America, the Caribbean, and northern South America (Sherry and Holmes 1997). This species is primarily insectivorous feeding on insects throughout the year. American Redstarts use small fruits and berries such as barberry (*Berberis* spp.), serviceberry (*Amelanchier* spp.), and magnolia (*Magnolia* spp.) in fall (Bent 1953, Martin et al. 1951) but only in very small quantities (< 5% fruits in fecal samples, Parrish 1997). As such, I classified American Redstarts as insectivorous.

1.3.3. American Robin (*Turdus migratorius*)

The American Robin is the most abundant and widespread species of thrush in North America and occupies both suburban and natural habitats (Sallabanks and James 1999). Their diet is highly variable and characteristically shifts from primarily hard-bodied and soft-bodied invertebrates during spring and summer to primarily fruits during autumn and winter. Based on the analyses of stomach contents of 1,169 robins collected across their range, fruits constituted > 90% of the diet in fall and winter, < 10% in spring, and intermediate amounts in summer (Wheelwright 1986). Over 50 different

genera of fruits and over 100 families of invertebrates have been documented in the diet of American Robins. Because their diet is typically a mixture of plant and animal material (White and Stiles 1990, Parrish 1997), robins are considered omnivores.

1.3.4. Baltimore Oriole (*Icterus galbula*)

The Baltimore Oriole, typically occupying woodland edges and open riparian woods, has also successfully adapted to urban and suburban landscapes (Rising and Flood 1998). It is commonly found in North America east of the Rocky Mountains during breeding season and winters from southern Mexico, throughout Central America, and into northern South America. Only a few orioles winter in southern United States (Rising and Flood 1998). This species feeds primarily on adult insects, particularly caterpillars, spiders and fruits during breeding season. In winter and migration, insects, fruits and nectar make up the diet of Baltimore Orioles (Timken 1970, Bent 1958). Fruits eaten include mulberries (*Morus* spp.), raspberries (*Rubus* spp.) and cherries (*Prunus* spp.) (Martin et al. 1951). There is very little quantitative data on the amounts of fruits this omnivorous species incorporates into its diet at different times of the year (see Timken 1970).

1.3.5. Black-and-White Warbler (*Mniotilta varia*)

Black-and-white Warblers occur throughout most of Canada and eastern and central United States during breeding season and southern Mexico, the Caribbean, Central America, and northwestern South America during winter (Kricher 1995). Most studies report no use of fruits at any time of the year for this species (Bent 1953, Blake and Loiselle 1992). However, Parrish (1997) found fruit material present in 9.0% of all fecal samples from this species. Nevertheless, the Black-and-white Warbler remained classified as an insectivore due to the overall lack of evidence of frugivory in their diet.

1.3.6. Cedar Waxwing (*Bombycilla cedrorum*)

The Cedar Waxwing, commonly found across Canada and the northern United States during summer and throughout much of the U.S and Mexico during winter, is best known for its dietary specialization on sugary fruits. This species occupies open

woodlands, riparian areas, orchards and suburban parks and gardens which have fruiting trees and shrubs (Witmer et al. 1997). Their diet consists primarily of fruits, especially during winter, with insects added during breeding season. Based on 65 years of data, Cedar Waxwings consume 84% fruits, 12% insects and 4% flower parts annually (Witmer 1996). This species is the only omnivorous passerine in North America known to include such a high proportion of fruits in its diet (Witmer 1996).

1.3.7. Common Yellowthroat (*Geothlypis trichas*)

One of the most widespread warblers throughout North America, the Common Yellowthroat breeds throughout Canada and the U.S., and winters primarily in Mexico, the Caribbean, and Central America. This species is generally found in thick vegetation in a wide range of habitats (Guzy and Ritchison 1999). Although one study found evidence of fruits in the diet of Common Yellowthroats (Parrish 1997), the majority of studies have only documented the presence of insects (see references in Guzy and Ritchison 1999) therefore this species was classified as an insectivore.

1.3.8. Gray Catbird (*Dumetella carolinensis*)

The Gray Catbird breeds throughout eastern and southern Canada and the U.S. and winters along the east coast of the U.S. to Mexico, into Central America and the Carribbean (Cimprich and Moore 1995). It is commonly found in dense, shrubby vegetation in natural habitats and sometimes residential areas. This omnivorous bird consumes both insects and small fruits from a variety of species (see references in Cimprich and Moore 1995). Annually, Gray Catbirds include varying percents of fruit in their diet: winter 76%, spring 20%, summer 60%, and fall 81% (Martin et al. 1951).

1.3.9. Hermit Thrush (*Catharus guttatus*)

The Hermit Thrush is widely distributed in North America and winters in southern U.S., through Mexico to Guatemala (Jones and Donovan 1996). Occupying a wide range of forested and edge habitats, this species is a terrestrial omnivore. During breeding, its diet consists mainly of insects and invertebrates, while it is heavily supplemented by a wide variety of fruits during migration and winter (Jones and

Donovan 1996). Although the percentage of animal and plant material in their diet varies with location and season, Martin et al. (1951) determined that, on a continental scale, the diet of Hermit Thrushes is made up of 93% and 40% animal matter in spring and winter, respectively, and Parrish (1997) found fruit material in 84% of fecal samples from Hermit Thrushes on fall migration.

1.3.10. House Wren (*Troglodytes aedon*)

The House Wren occurs in southern Canada and much of the U.S. during breeding and winters in the southern U.S. and Mexico (Johnson 1998). Commonly found in open, shrubby woodlands, including suburban backyards and city parks, this species feeds primarily on terrestrial invertebrates (see references in Johnson 1998). Although some fruit material was found in fecal samples of House Wrens on fall migration (Parrish 1997), I classified this species as an insectivore because most of the literature did not document the presence of fruits in their diet.

1.3.11. Least Flycatcher (*Empidonax minimus*)

The Least Flycatcher breeds across Canada and the northern U.S. in deciduous or mixed forests and winters in southern Mexico into Central America (Briskie 1994). The summer diet is composed of almost exclusively insects with fruits and seeds taken occasionally (e.g. raspberries (*Rubus* spp.) and elderberries (*Sambucus canadensis*; Beal 1912 in Briskie 1994). Winter diet has not been quantified but is assumed to be primarily insectivorous as well. During fall migration, fruit material was present in 53% of fecal samples from Least Flycatchers (Parrish 1997). As such, the Least Flycatcher was classified as an omnivore.

1.3.12. Magnolia Warbler (*Dendroica magnolia*)

The Magnolia Warbler is commonly found in Canada's Boreal forest and some of the eastern states during breeding and into southern Mexico, Central America and the Caribbean during winter (Hall 1994). This species feeds on insects, particularly lepidopteran caterpillars, during breeding season (Kendeigh 1947 and Crawford et al. 1983 in Hall 1994). There is no information available on its winter diet. Tramer and

Tramer (1977) observed Magnolia Warblers feeding on *Lonicera* sp. berries during migration but only in inclement weather. I am only aware of one study that has documented a significant proportion of fruit in the diet of this species (Parrish 1997). Nevertheless, I classified the Magnolia Warbler as an insectivore because, overall, the available data indicated little to no fruits in its diet.

1.3.13. Orange-crowned Warbler (*Vermivora celata*)

Commonly found throughout most of western and northern North America and Canada's eastern Boreal forest, the Orange-crowned Warbler is an omnivorous bird feeding primarily on insects during breeding season (Sogge et al. 1994). During winter, in the southern U.S., Mexico and Central America, this species eats fruits and nectar in addition to insects, and will be attracted to feeders with suet. There have been few quantitative studies. Beal (1907, in Sogge et al. 1994) reported 91% animal matter and 9% plant matter in the diet of Orange-crowned Warblers but sample dates were not given.

1.3.14. Song Sparrow (*Melospiza melodia*)

The Song Sparrow is widespread across North America in breeding season and throughout the United States during winter in a wide range of forest, shrub and riparian habitats (Arcese et al. 2002). Truly omnivorous, this species feeds on insects and invertebrates, seeds and fruits during breeding and non-breeding periods but in varying proportions (see references in Arcese et al. 2002). The amount of plant material in its diet varies from 86% in winter, 54% in spring, 60% in summer and 92% in fall (Martin et al. 1951). Parrish (1997) documented 49% fruits in the diet of Song Sparrows during fall migration.

1.3.15. Tree Swallow (*Tachycineta bicolor*)

The Tree Swallow breeds throughout central and northern North America, usually in open areas near water, and winters primarily in Florida and along Gulf of Mexico (Robertson et al. 1992). Its diet consists mainly of flying insects throughout the year but also includes fruits and seeds during winter and inclement weather (see

references in Robertson et al. 1992). Fruits in their diet are usually bayberries (*Myrica* spp.) allowing them to winter as far north as eastern Massachusetts (Place and Stiles 1992). An overall diet of 80% animal material and 20% plant material has been documented (Beal 1918 in Robertson et al. 1992), therefore the Tree Swallow was classified as an omnivore.

1.3.16. Warbling Vireo (*Vireo gilvus*)

The Warbling Vireo has a broad breeding distribution including southern Canada north into Alberta and British Columbia and much of the north-central United States, and winters in western Mexico and northern Central America (Gardali and Ballard 2000). Although it occupies a variety of deciduous forest habitats, this species has adapted well to human landscapes such as urban parks, orchards and farm fencerows. Warbling Vireos are omnivorous birds feeding primarily on insects throughout the year and including some fruits in its winter diet. However, the majority of fruits are taken in fall (Chapin 1925 in Gardali and Ballard 2000, Parrish 1997).

1.4. THESIS OBJECTIVES AND ORGANIZATION

The general objectives of my thesis were as follows:

1) To evaluate the effectiveness of the stable isotope technique to differentiate between insectivorous and omnivorous migratory songbirds using blood and claw $\delta^{13}C$ and $\delta^{15}N$ values.

2) To use blood and claw $\delta^{13}C$ and $\delta^{15}N$ measurements from a diverse group of migratory songbirds to examine within-species diet shifts using seasonal differences in stable isotope values.

3) To examine seasonal shifts in frugivory of a known omnivorous songbird, the Yellow-rumped Warbler, using stable isotope analysis of tissues representing different periods of dietary history.

Even though stable isotope analysis has had much success in various dietary studies in recent years, this is the first study to use this technique to investigate frugivory

in temperate migratory songbirds. Testing the correlation between dietary information of different tissues by regressing stable isotope signatures among tissues was also a unique contribution; this had not yet been examined in wild birds, only in captive individuals. Since metabolic rates of captive and wild birds can differ, this objective allowed me to compare the findings of captive studies to wild birds as values derived from these studies are applied to wild birds and assumed to be a fairly accurate reflection of natural processes. Throughout this thesis, the term "omnivorous" is used to refer to birds which feed on both fruits and insects as opposed to insectivores which feed only on insects. My thesis is organised into four chapters. Chapters 1 and 4 cover the general introduction and the summary and synthesis, respectively. Chapter 2, *Stable isotope tracking of frugivory in migratory passerines*, focuses on all sixteen study species and addresses objectives 1 and 2. Chapter 3, *Annual diet variations in Yellow-rumped Warblers: a stable isotope investigation*, focuses on Yellow-rumped Warblers and addresses objective 3. Chapters 2 and 3 are structured as journal articles. The first appendix presents data regarding the breeding and wintering catchment areas of Yellow-rumped Warblers migrating through the DMBO. The second appendix presents supplemental tables for Chapter 2.

CHAPTER 2: STABLE ISOTOPE TRACKING OF FRUGIVORY IN MIGRATORY PASSERINES

2.1. INTRODUCTION

Dietary plasticity, involving a shift from an insect diet during the breeding season to one incorporating fruit, berries and other plant materials during migration and non-breeding periods, has been documented in several species of North American migratory songbirds (Wheelwright 1988, White and Stiles 1990, Parrish 1997, Parrish 2000). This behavior is one of several adaptations associated with migration especially in temperate migrant songbirds (Berthold 2001). In temperate zones, the dietary shift to fruits occurs mainly during fall migration because insect abundance diminishes and most fruit species become abundant and readily accessible then (Johnson et al. 1985, White and Stiles 1990, Parrish 1997, Suthers et al. 2000, Smith et al. 2007). For some species, frugivory continues on wintering grounds in the tropics (e.g., Greenberg 1981, Blake and Loiselle 1992, Levey and Stiles 1992, Prather 2000, Herrera et al. 2005) or the temperate zone (e.g., Parrish 2000, Borgmann et al. 2004, Kwit et al. 2004a and 2004b). Frugivory during spring migration within the temperate zone, involving birds using fruits that remained from the previous fall and persisted through winter, has also been documented in some species (Willson 1991; see references within Parrish 2000) but frugivory during spring migration remains understudied. While frugivory in fall migrants may reflect a change in food availability (Bairlein 1990, Berthold 2001), seasonal diet shifts may also be associated with inherent changes in food preference and/or nutritional requirements (Bairlein 1990, Bosque and Pacheco 2000, Gannes 2001, Bairlein 2002) as the choice of fruit is often species-specific and not always related to local fruit abundance (Greenberg 1981, Malmborg and Willson 1988, Kwit et al. 2004a) and fruits represent a food source that may help with fat deposition necessary for migrants (Moermond and Denslow 1985, Bairlein 1996).

Frugivory in omnivorous migrant songbirds is known to contribute to seed dispersal of temperate plants (e.g., Snow 1970, Willson 1986, Malmborg and Willson 1988) but the extent of frugivory in North American migratory songbirds is not well known. To date, few studies have been conducted in temperate zones to evaluate diet variations in omnivorous migratory songbirds throughout their annual cycle (Parrish 1997, Parrish 2000, Suthers et al. 2000). In addition to being important in terms of understanding the evolution of migration in North American avifauna, establishing dietary and habitat requirements of migratory songbirds has implications for the conservation and management of songbird populations and their habitats throughout their annual cycle (Moore et al. 1995, Yong et al. 1998, Hutto 2000, Petit 2000).

Previous studies have evaluated frugivory in songbirds using traditional methods, such as fecal or stomach-content analyses (e.g., Johnson et al. 1985, White and Stiles 1990, Blake and Loiselle 1992, Parrish 1997, Prather 2000). Assessing diets through conventional techniques are effective and often non-destructive. However, the use of fecal samples can be problematic due to differential rates of passage and processing of food types resulting in a possible biased representation of ingested food items (Rosenberg and Cooper 1990). Similarly, these major disadvantages can apply to stomach content analyses through collections (Rosenberg and Cooper 1990) or the use of emetics (Tomback 1975, Gavett and Wakeley 1986) with the additional problem that individuals with empty stomachs provide no information on types of food eaten.

Stable isotope analysis has become an important tool for assessing avian diets. Several studies have used stable isotopes to examine trophic relationships (Hobson 1999b, Herrera et al. 2003); infer feeding habitats (Marra et al. 1998, Wassenaar and Hobson 2000); and diet (Alisauskas and Hobson 1993, Podlesak et al. 2005). Stable isotope analysis can be non-destructive if tissue samples such as feathers, blood, or claws are used. Because stable isotope ratios of elements are measured in tissues of consumers, the use of this technique augments traditional methods by providing information on assimilated vs. recently ingested foods, over a period of days to months depending on the choice of tissues (Hobson and Clark 1992a, Evans-Ogden et al. 2004). In small passerines, whole blood and claws reliably integrate diet over a period of 2-3

weeks and 2-3 months, respectively (Bearhop et al. 2003, Hobson and Bairlein 2003, Pearson et al. 2003).

Consumers typically become enriched in ^{15}N (measured as $\delta^{15}N$) with increasing trophic level due to the preferential excretion of the lighter isotope ^{14}N during protein synthesis and catabolism (Peterson and Fry 1987). Enrichment in ^{15}N ranges from about 2.5 to 5‰ for each increase in trophic level (Kelly 2000, Peterson and Fry 1987) with an average of about 3.4 ‰ (Kelly 2000, Post 2002). Therefore, it is possible to detect diet shifts across trophic levels (e.g., herbivory vs. insectivory) by quantifying $\delta^{15}N$ variations in tissues of consumers (e.g. Herrera et al. 2001a, Herrera et al. 2003). Previous work has also shown slight increases in food web $\delta^{13}C$ values with trophic level (Peterson and Fry 1987) as well as decreasing $\delta^{13}C$ values in plants with increasing latitude in North America (Körner et al. 1991).

Omnivorous diets in terrestrial consumers have been examined using stable isotope analysis in some mammals (Hobson and Stirling 1997, Herrera et al. 2001a, 2001b). However, few studies have examined isotopically frugivory in birds and these have predominately been carried out in the tropics (Prather 2000, Herrera et al. 2003, Herrera et al. 2005) or in captive studies (Hobson and Bairlein 2003, Pearson et al. 2003). Hobson (1999b) alluded to the usefulness of stable carbon and nitrogen isotope analysis for investigating the degree of frugivory in birds although that study was not specifically designed to investigate frugivory. However, with the exception of Podlesak et al.'s (2005) study of Yellow-rumped Warblers, Golden-crowned Kinglets (*Regulus satrapa*), Ruby-crowned Kinglets (*Regulus calendula*), White-throated Sparrows (*Zonotrichia albicollis*) and Gray Catbirds migrating along the eastern seaboard of North America in the Fall, few if any studies have examined isotopically the occurrence of frugivory in migrant North American passerines, either during migration or on the wintering grounds.

My objectives were to use $\delta^{13}C$ and $\delta^{15}N$ measurements of blood and claws of migrating passerines to: 1) evaluate the usefulness of the isotope approach to differentiate between insectivorous and omnivorous migratory songbirds and 2) examine diet shifts within species using seasonal differences in stable isotope values. Since insectivores in general feed at higher trophic levels than omnivores, I predicted that

tissues of obligate insectivorous songbirds would have higher $\delta^{15}N$ values than omnivores. Furthermore, I expected insectivores to show lower variation in $\delta^{15}N$ values between seasons while omnivorous species were expected to have higher $\delta^{15}N$ values in summer compared to winter and higher $\delta^{15}N$ values during spring migration compared to fall migration because it has been documented that fruits dominate fall migration and winter diet (Parrish 1997, Suthers et al. 2000, Borgmann et al. 2004). Stable-carbon isotope values were expected to be less useful for detecting trophic shifts because of the low trophic-enrichment of $\delta^{13}C$ but potential latitudinal information (e.g., higher $\delta^{13}C$ values with decreasing latitude) was of interest. I examined these questions using stable isotope measurements from a broad range of species during spring and fall migration.

2.2. METHODS

2.2.1. Study Area

During spring and fall migration of 2003, avian tissue collections were made at the Delta Marsh Bird Observatory (DMBO), located in the dune-ridge forest of Delta Marsh, Manitoba, Canada (98°23' W, 50°11' N) (MacKenzie 1982). This forest, which abuts the south end of Lake Manitoba, is a primary stopover site for migratory songbirds *en route* to and from their breeding and wintering grounds (Mazerolle et al. 2005).

2.2.2. Study Species

I sampled sixteen species of songbirds intercepted during migration (Table 2.1). These species were chosen to represent a range of dietary habits incorporating varying amounts of fruits and insects. Knowledge of each species' diet was based largely on corresponding accounts in Birds of North America (Poole 2005; Table 2.1) and recent quantitative studies (Parrish 1997). Species were subsequently classified in insectivore or omnivore (i.e., fruit and insect diet) guilds.

2.2.3. Avian sampling

Songbirds were captured during spring (1 – 27 May 2003) and fall migration (1 August to 30 September 2003) using standard constant-effort mist netting and banding protocols (Hussell and Ralph 1998). I aimed to collect blood and claw samples

Table 2.1 Species sampled at the Delta Marsh Bird Observatory, MB, during spring and fall migration 2003, scientific name, species code, and sample sizes for blood and claw tissues. Diet classification assigned to each species was determined based on information found in their respective Birds of North America accounts.

Species	Scientific Name	Code	Blood		Claws		Reference
			Spring	Fall	Spring	Fall	
INSECTIVORES							
American Redstart	*Setophaga ruticilla*	AMRE	7	10	9	10	Sherry and Holmes 1997
Black-and-white Warbler	*Mniotilta varia*	BAWW	4	11	5	11	Kricher 1995
Common Yellowthroat	*Geothlypis trichas*	COYE	12	10	17	10	Guzy and Ritchison 1999
House Wren	*Troglodytes aedon*	HOWR	9	11	9	11	Johnson 1998
Magnolia Warbler	*Dendroica magnolia*	MAWA	5	10	10	10	Hall 1994
OMNIVORES							
American Robin	*Turdus migratorius*	AMRO	12	2	13	3	Sallabanks and James 1999
Baltimore Oriole	*Icterus galbula*	BAOR	10	5	11	6	Rising and Flood 1998
Gray Catbird	*Dumetella carolinensis*	GRCA	10	8	10	10	Cimprich and Moore 1995
Cedar Waxwing	*Bombycilla cedrorum*	CEDW	9	2	12	3	Witmer et al. 1997
Hermit Thrush	*Catharus guttatus*	HETH	15	10	15	10	Jones and Donova 1996
Least Flycatcher	*Empidonax minimus*	LEFL	15	10	17	10	Briskie 1994
Yellow-rumped Warbler	*Dendroica coronata*	YRWA	15	10	15	10	Hunt and Flaspohler 1998
Orange-crowned Warbler	*Vermivora celata*	OCWA	10	10	12	10	Sogge et al. 1994
Song Sparrow	*Melospiza melodia*	SOSP	10	11	9	11	Arcese et al. 2002
Tree Swallow	*Tachycineta bicolor*	TRES	10	10	10	10	Robertson et al. 1992
Warbling Vireo	*Vireo gilvus*	WAVI	10	10	10	10	Gardali and Ballard 2000

from 10-15 birds per species. Because of the relatively short dietary integration period of 2 to 3 weeks (Hobson and Bairlein 2003, Pearson et al. 2003), $\delta^{15}N$ and $\delta^{13}C$ values from whole blood were assumed to reflect diet during spring and fall migration periods. Conversely, claws of small passerines likely integrate diet over 2 to 3 months (Bearhop et al. 2003) and, therefore, $\delta^{15}N$ and $\delta^{13}C$ values from claws sampled in spring and fall were assumed to reflect diet during late winter and mid-late summer, respectively. Blood samples were collected from the brachial vein using a sterilized needle (27-gauge) and a heparanized capillary tube (full volume = 70 μL) then preserved in 70% ethanol (Campbell 1995, Hobson et al. 1997). The volume of blood collected was approximately 35 μL (i.e., about half of a capillary tube) or less. Claws were clipped from both the left and right inner and middle toe of all sampled birds using nail clippers. Growth rates of different claws were not expected to vary within the same individual (Bearhop et al. 2003) and so they were selected at random. Approximately 2 to 3 mm of nail from the distal end of the claw was clipped. None of the species surveyed in this study are ground foragers so claws should not wear down faster than the average amount of claw wear expected for songbirds; as such, I assumed that the entire part of the claw sampled would adequately represent breeding and wintering ground diets (Bearhop et al. 2003, Mazerolle and Hobson 2005). Finally, morphometric measurements were taken and individuals were aged and sexed using species-specific criteria outlined in Pyle (1997).

2.2.4. Fruit and Insect Sampling

Sampling of foodweb components was conducted to estimate the isotopic range of food sources available to migrants associated with the terrestrial environment in the fall. Fruits and insects were sampled from southern agricultural areas and boreal forest. In fall, my avian sample consisted of birds originating from boreal, aspen parkland and agricultural regions. For the aspen parkland and agricultural regions, ripe fruits were collected between 26 July and 26 September, 2003, at Delta Marsh, Manitoba, and Redberry Lake (107º09' W, 52º43' N) and Saskatoon (106º39' W, 52º07' N), Saskatchewan. Boreal sites included Christopher Lake (105º49' W, 53º33' N) and Emma Lake (105º53' W, 53º35' N), Saskatchewan.

Table 2.2 Mean (± SD), range, percent N and C, and C/N ratios for $\delta^{15}N$ and $\delta^{13}C$ values of fruit species collected between July 26 and September 26, 2003 at various locations within boreal and agriculture landscapes.

Species	Scientific Name	Location	n	$\delta^{15}N$ (‰)	Range	$\delta^{13}C$ (‰)	Range	%N	%C	C/N ratio
Boreal										
Baneberry	*Actaea rubra*	Emma Lake, SK	1	1.2		-28.0		0.9	40.3	46.6
Bunchberry	*Cornus canadensis*	Christopher Lake, SK	1	1.1		-27.2		0.6	39.9	69.1
		Emma Lake, SK	1	-0.01		-26.3		0.7	40.6	62.9
Canada Buffaloberry	*Shepherdia canadensis*	Christopher Lake, SK	1	0.5		-25.7		1.7	43.0	25.5
Choke Cherry	*Prunus virginiana*	Christopher Lake, SK	1	1.3		-28.1		0.6	42.4	67.2
		Emma Lake, SK	1	5.2		-27.3		0.7	44.5	62.7
Low-bush cranberry	*Viburnum edule*	Christopher Lake, SK	1	-0.3		-25.6		0.2	40.5	167.5
		Emma Lake, SK	1	0.2		-26.7		0.2	39.7	172.9
Pin Cherry	*Prunus pensylvanica*	Christopher Lake, SK	2	-2.1 ± 0.3	-2.3 - -1.9	-25.7 ± 0.2	25.7 - -25.5	0.4	40.2	116.5
		Emma Lake, SK	1	0.6		-24.5		0.6	39.2	64.8
Prickly Rose	*Rosa acicularis*	Christopher Lake, SK	1	1.6		-25.2		1.2	39.6	34.1
		Emma Lake, SK	1	7.4		-24.2		1.3	41.2	31.2
Red-osier Dogwood	*Cornus stolonifera*	Christopher Lake, SK	1	-1.6		-29.4		0.9	52.4	58.5
		Emma Lake, SK	1	-1.3		-28.5		0.7	57.6	78.4
Saskatoon	*Amelanchier alnifolia*	Christopher Lake, SK	1	0.9		-27.1		0.4	40.3	102.1
Wild Red Raspberry	*Rubus idaeus*	Emma Lake, SK	1	3.3		-25.2		1.1	40.5	37.3
Agriculture										
Bittersweet	*Solanum dulcamara*	Delta Marsh, MB	2	7.0 ± 0.3	6.8 – 7.2	-26.1 ± 1.4	-27.1 - -25.1	3.1	44.5	14.4
Choke Cherry	*Prunus virginiana*	Delta Marsh, MB	3	4.8 ± 2.5	1.8 – 6.3	-25.6 ± 0.7	-26.3 - -24.9	0.7	40.1	56.5

Common Snowberry	*Symphoricarpos albus*	Saskatoon, SK	4	1.3 ± 4.3	-5.0 – 4.1	-26.2 ± 1.7	-27.7 - - 24.1	0.7	41.1	59.1
		Delta Marsh, MB	1	4.1		-25.8		0.6	45.6	75.5
		Saskatoon, SK	2	4.8 ± 3.0	2.7 – 6.9	-23.8 ± 0.7	-24.3 - - 23.4	0.5	41.8	82.0
Gooseberry		Redberry Lake, SK	1	7.4		-23.4		0.7	38.7	55.8
Low-bush cranberry	*Viburnum edule*	Delta Marsh, MB	1	2.4		-22.9		0.3	41.8	141.2
Prickly Rose	*Rosa acicularis*	Delta Marsh, MB	2	5.9 ± 1.0	5.2 – 6.7	-24.2 ± 0.1	-24.2 - - 24.1	0.5	42.4	87.3
Red-osier Dogwood	*Cornus stolonifera*	Delta Marsh, MB	2	4.5 ±2.9	2.4 – 6.5	-33.1 ± 6.8	-37.9 - - 28.3	1.2	49.4	41.2
Saskatoon	*Amelanchier alnifolia*	Saskatoon, SK	2	0.8 ± 4.1	-2.1 – 3.7	-27.6 ± 0.5	-28.0 - - 27.2	0.8	46.4	59.1
		Redberry Lake, SK	1	2.6		-24.9		0.5	38.3	82.9
Silver Buffaloberry	*Shepherdia argentea*	Saskatoon, SK	3	-0.8 ± 1.2	-2.3 - - 0.1	-25.6 ± 1.5	-27.2 - - 24.4	0.4	40.2	105.8
		Redberry Lake, SK	1	-0.4		-26.8		1.1	45.9	41.5
Tartarian Honeysuckle	*Lonicera tartarica*	Saskatoon, SK	2	-0.7 ± 0.3	-0.9 - - 0.5	-26.3 ± 2.4	-28.0 - - 24.7	1.0	43.7	42.9
		Saskatoon, SK	4	-2.7 ± 0.8	-3.7 - - 1.9	-26.6 ± 0.8	-27.3 - - 25.5	0.4	40.5	94.1
Virginia Creeper	*Parthenocissus quinquefolia*	Delta Marsh, MB	2	7.9 ± 2.8	5.9 – 9.8	-27.9 ± 0.1	-28.0 - - 27.8	0.8	50.8	62.7
Western Mountain Ash	*Sorbus scopulina*	Saskatoon, SK	1	6.4		-23.2		4.3	45.1	10.5

I collected 56 samples of fruits representing the genera *Actaea*, *Amelanchier*, *Cornus*, *Lonicera*, *Parthenocissus*, *Prunus*, *Rosa*, *Rubus*, *Sheperdia*, *Solanum*, *Sorbus*, *Symphoricarpos*, and *Viburnu*m (Table 2.2). Since they are not typically digested by birds, seeds and skin were removed and the fleshy part of each fruit was retained for analysis. Approximately 5 to 60 berries, depending on their size, were combined for each sample to have an adequate amount of material for analysis.

Insects were not sampled as a part of this study but stable isotope data from a previous study were used (Bennett and Hobson, unpublished data). Insects were collected from May to August 2002 and 2003, in Prince Albert National Park (53°50′ N, 105°50′ W), Saskatchewan, and identified to Order. For my study, data representing the orders Araneae, Coleoptera, Diptera, Hymenoptera, Lepidoptera, Odonata, and Orthoptera were used (Table 2.3).

2.2.5. Stable Isotope Analysis

Ethanol was decanted from blood samples and blood was freeze dried. Claws were cleaned of surface oils with a 2:1 chloroform:methanol mixture and allowed to air dry. Fruit samples were freeze dried and ground to a fine powder using a small mortar and pestle. For consistency, five replicates of each fruit sample were used to generate an average value and eliminate any biases related to potential variance in sample homogeneity.

Approximately 1mg of dry blood, claw and insect material and 7mg of fruit material were subsampled for each isotopic measurement. Sample materials were sealed into tin cups and combusted at 1200ºC in a Robo-Prep elemental analyzer. The resultant CO_2 and N_2 gases were then analyzed using a Carlo Erba elemental analyzer interfaced with a Europa 20:20 continuous flow isotope ratio mass spectrometer (CFIRMS). Laboratory standards of egg albumen and whale baleen were used for blood, claw and insect samples, and a peagrain standard was used for fruit samples.

Samples were analyzed at the Soil Science Laboratory, University of Saskatchewan, Saskatoon, Saskatchewan. Stable isotope measurements were expressed as parts-per-thousand (‰) deviations from a known standard. All values are reported in δ (delta) notation according to:

Table 2.3 Sample size (n), mean (± SD), range, and percent N and C for $\delta^{15}N$ and $\delta^{13}C$ values for herbivorous and predaceous terrestrial arthropods sampled in Prince Albert National Park, SK between June and August 2003. Insects are categorized at the Order level.

Arthropod Order	n	$\delta^{15}N$ (‰)	Range	$\delta^{13}C$ (‰)	Range	%N	%C
Herbivorous							
Coleoptera	18	3.7 ± 4.1	-2.8 to 9.4	-23.7 ± 1.1	-26.5 to -22.5	10.1	46.5
Diptera	6	4.3 ± 2.4	1.6 to 6.7	-27.7 ± 3.1	-31.1 to -23.8	10.5	41.8
Hymenoptera	3	1.7 ± 1.5	0.07 to 2.8	-25.1 ± 0.8	-25.7 to -24.3	12.8	52.2
Lepidoptera	39	2.8 ± 1.9	-0.9 to 6.6	-28.0 ± 1.9	-32.4 to -24.2	10.8	51.4
Orthoptera	16	2.5 ± 1.5	-0.02 to 5.6	-25.6 ± 1.0	-26.7 to -23.8	12.7	49.6
Predaceous							
Araneae	6	6.8 ± 0.8	6.1 to 8.2	-25.7 ± 1.4	-27.9 to -24.2	13.1	51.0
Coleoptera	45	6.2 ± 2.1	0.2 to 10.2	-25.2 ± 1.3	-29.8 to -22.9	10.1	50.9
Diptera	4	5.5 ± 1.7	4.0 to 7.7	-26.9 ± 1.7	-29.1 to -25.2	13.3	50.0
Hymenoptera	9	4.6 ± 2.0	2.3 to 7.8	-24.3 ± 1.2	-26.1 to -22.6	12.5	52.8
Odonata	32	6.2 ± 1.4	3.5 to 8.2	-26.2 ± 2.4	-31.8 to -23.0	12.8	53.3

$$\delta x = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (2.1)$$

where R_{sample} is the isotope ratio of the sample and $R_{standard}$ is the isotope ratio of the standard. International isotopic standards used for carbon and nitrogen were Vienna Pee Dee Belemnite (VPDB) and atmospheric Air, respectively. Analytical measurement error was estimated to be ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$ measurements.

2.2.6. Statistical Analysis

I evaluated effects of species, season, and capture day as predictors of blood and claw $\delta^{13}C$ and $\delta^{15}N$ values using general linear models (GLM). The same models were subsequently evaluated using diet guilds instead of species. All models were run separately for both tissues and isotopes because tissues represent different timeframes and different responses were expected with each tissue and isotope combination. Specifically, I evaluated models including all three variables independently, as well as models with all additive combinations of these variables and two interactions. Akaike's Information Criterion, corrected for small sample size (AIC_c), was used to rank competing models (Burnham and Anderson 2002). The model with the lowest AIC_c value was considered to be the most parsimonious, given the data and the candidate set of models. To compare competing models, I computed ΔAIC_c by calculating the difference in AIC_c scores between a given model and the model with the lowest AIC_c. Burnham and Anderson (2002) outlined that models with a ΔAIC_c score of 0-2, 4-7, and >10 provide substantial, little, or no support, respectively. I then calculated Akaike weights which provide a measure of the weight of evidence for each model (e.g., a model with an Akaike weight of 0.70 has a 70% chance of being the most parsimonious model; Anderson et al. 2000). Finally, I assessed the relative importance (RI) of individual predictor variables (i.e., species, season, and day) by summing the Akaike weights across all models in the set in which a variable occurred.

Within-species seasonal differences in blood and claw $\delta^{13}C$ and $\delta^{15}N$ values were evaluated using least-square means and 95% confidence intervals. Also, blood and claw $\delta^{13}C$ and $\delta^{15}N$ values were compared between age and sex classes within each species with two-tailed, two-sample t-tests.

Multiple-Response Permutation Procedures (MRPP) were used to assess the significance of differences in stable isotope values between fruit and insect groups defined *a priori* (Agriculture fruits, Boreal fruits, Herbivorous insects, and Predaceous insects) using PC-ORD (McCune and Mefford 1999). Analyses used ranked data and relative Euclidean distance. MRPP provides a test statistic (T), which describes the separation between groups, a P value and a measure of effect size (A) (i.e., within group homogeneity). A more negative number for T represents a larger difference between groups; conversely, a high A value indicates a higher degree of similarity between samples within a group (McCune and Grace 2002).

I evaluated associations of blood and claw $\delta^{15}N$ and $\delta^{13}C$ values with capture day, within each species, using linear regression analysis. Partial regression analysis was used to examine relations of blood and claw $\delta^{15}N$ and $\delta^{13}C$ values with body condition, also within species, while controlling for effects of capture day. Body condition was calculated by dividing body mass by unflattened wing length cubed (g/mm^3) and then multiplying by 10,000 to facilitate calculations (Winker et al. 1992).

2.3. RESULTS

2.3.1. Modeling Results for $\delta^{15}N$ and $\delta^{13}C$

Among the candidate set of models describing $\delta^{15}N$ values of blood and claws, the top four models had ΔAIC_c values < 2. The most parsimonious models included species, season, and an interaction between these two variables and carried 41% and 43% of the AIC_c weights, respectively. The second-ranked models for blood and claw $\delta^{15}N$ values included the addition of capture day and provided 16% and 17% support, respectively (Table 2.4). The relative importance of variables indicated that species was the most important variable in the set (RI=1.0) for models describing both blood and claw $\delta^{15}N$ values, followed by season (blood: RI=0.83, claw: RI=0.89) and day (blood: RI=0.59, claw: RI=0.57). Models with diet guild (i.e., insectivore vs. omnivore) provided no additional support. This indicates that species alone explained more of the variation in $\delta^{15}N$ values than species classified by diet guilds. Indeed, there was no clear segregation in $\delta^{15}N$ values between species I classified as insectivores and omnivores

Table 2.4 Results of Akaike criteria for competing general linear models for blood and claw $\delta^{15}N$ and $\delta^{13}C$ values of sixteen migratory songbirds. Models are based on $\delta^{15}N$ and $\delta^{13}C$ values of 303 individuals for blood and 329 individuals for claws. Birds were captured at the Delta Marsh Bird Observatory, MB, during spring and fall migration of 2003. Models are ranked using Akaike's Information Criterion adjusted for sample size (AIC_c). K = number of parameters, ΔAIC_c = difference in AIC_c between a given model and the model with the lowest AIC_c, weight = Akaike weight.

MODELS	K	AIC_c	ΔAIC_c	weight
Blood $\delta^{15}N$				
species, season, species*season	33	118.89	0.00	0.41
species, season, day, species*season	34	119.88	0.99	0.25
species, day, species*day	33	120.62	1.74	0.17
species, season, day, species*day	34	120.69	1.81	0.17
Blood $\delta^{13}C$				
guild, season	4	-5.70	0.00	0.16
species, season	18	-5.56	0.14	0.15
species, season, day	19	-5.31	0.39	0.14
season	3	-4.28	1.41	0.081
Claws $\delta^{15}N$				
species, season, species*season	33	151.87	0.00	0.43
species, season, day, species*season	34	152.85	0.98	0.26
species, season, day, species*day	34	153.47	1.60	0.19
species, day, species*day	33	154.60	2.73	0.11
Claws $\delta^{13}C$				
species, season, day	19	68.47	0.00	0.31
species, season	18	70.24	1.77	0.13
season	3	70.51	2.04	0.11
species, season, species*season	33	72.21	3.74	0.048

(Figure 2.1). Season appeared in three of the four top models and accounted for a moderate amount of variability in $\delta^{15}N$ values. There was considerable variability in species $\delta^{15}N$ values for both tissues (blood and claws) and sampling seasons (spring and fall) (Figure 2.1).

Among the models describing $\delta^{13}C$ variation of blood and claws, none of the models showed strong explanatory power (Table 2.4). For $\delta^{13}C$ values of blood, the top three models provided similar amounts of support with 16%, 15%, and 14%, respectively. The top two models were guild and season, and species and season. However both provided roughly the same amount of low support indicating that neither guild nor species are strong explanatory variables for blood $\delta^{13}C$ values. The common predictor variable among all three models was season. For $\delta^{13}C$ values of claws, the most parsimonious model included species, season, and day and carried 31% of the Akaike weights. All remaining models in the candidate set each provided less than 15% of the support. For models evaluating $\delta^{13}C$ variation, the most important variable in the set was season (blood: RI=0.92, claw: RI=0.97) followed by species (blood: RI=0.69, claw: RI=0.76) and day (blood: RI=0.46, claw: RI=0.60). None of the models including these three variables provided any substantial support suggesting that the majority of the variation in $\delta^{13}C$ values was attributable to other variables. Stable-carbon isotope values of blood and claws within seasons were not significantly different between species and indicated that birds were feeding in a terrestrial C_3 plant-based food web. Also, the range of $\delta^{13}C$ values was narrower during summer (Fig. 2.1D) and fall migration (Fig. 2.1B) suggesting a greater similarity in habitat use during these periods relative to winter and spring migration.

2.3.2. Seasonal Differences

Within-species seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ values varied according to tissue and guild (Table 2.5). For blood $\delta^{15}N$ values, insectivores did not show significant seasonal differences, as predicted, with the exception of House Wrens which had higher $\delta^{15}N$ values during fall vs. spring migration and Magnolia Warblers which had lower $\delta^{15}N$ values during fall vs. spring migration. Some omnivorous species (Baltimore Oriole, Gray Catbird, Least Flycatcher, Warbling Vireo) showed an increase in blood

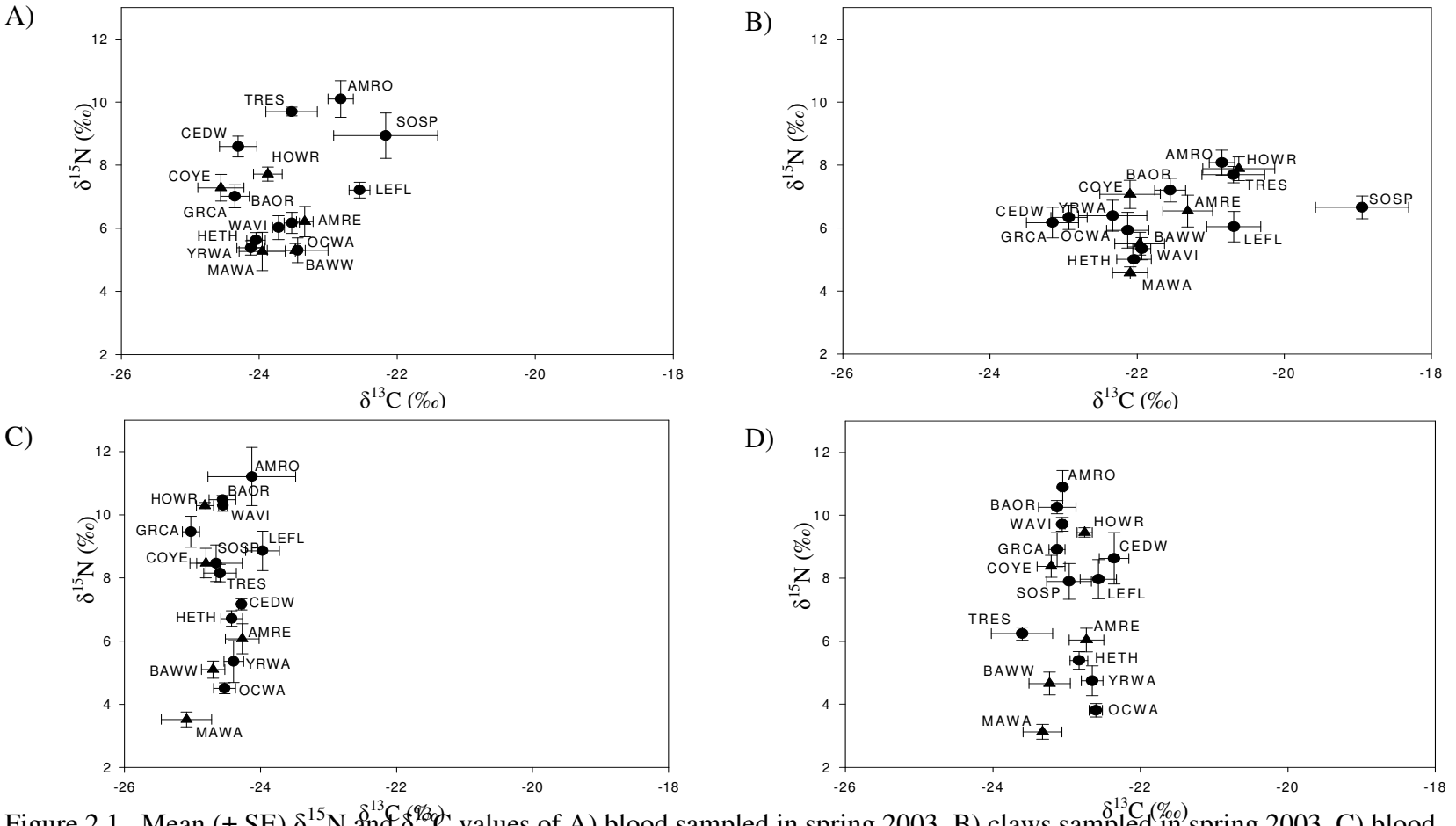


Figure 2.1 Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ values of A) blood sampled in spring 2003, B) claws sampled in spring 2003, C) blood sampled in fall 2003, and D) claws sampled in fall 2003 for insectivorous (triangles) and omnivorous (circles) songbirds captured at the Delta Marsh Bird Observatory. A species' full name corresponding to its 4-letter code and tissue sample sizes for each species can be found in Table 2.1.

Table 2.5 Least square means and 95% confidence intervals (CI) for seasonal differences in $\delta^{15}N$ and $\delta^{13}C$ values of blood and claws. Tissues were sampled from species migrating through the Delta Marsh Bird Observatory, MB, during spring and fall of 2003. See Table 2.1 for a species' full name corresponding with its 4-letter code.

Species	Season	n	$\delta^{15}N$ (‰)	95% CI		$\delta^{13}C$ (‰)	95% CI	
Blood				Lower	Upper		Lower	Upper
AMRE	Spring	7	6.2	5.3	7.1	-23.3	-24.0	-22.7
	Fall	10	6.1	5.3	6.9	-24.3	-24.8	-23.7
AMRO	Spring	12	10.1	9.4	10.8	-22.8	-23.3	-22.3
	Fall	2	11.2	9.5	13.0	-24.1	-25.3	-22.9
BAOR*	Spring	10	6.2	5.4	6.9	-23.5	-24.1	-23.0
	Fall	5	10.5	9.4	11.6	-24.6	-25.3	-23.8
BAWW	Spring	4	5.3	4.1	6.5	-23.5	-24.3	-22.6
	Fall	11	5.1	4.4	5.8	-24.7	-25.2	-24.2
CEDW	Spring	9	8.6	7.8	9.4	-24.3	-24.9	-23.7
	Fall	2	7.2	5.4	8.9	-24.3	-25.5	-23.1
COYE	Spring	12	7.3	6.6	8.0	-24.6	-25.0	-24.1
	Fall	10	8.5	7.7	9.2	-24.8	-25.3	-24.3
GRCA*	Spring	10	7.0	6.2	7.8	-24.4	-24.9	-23.8
	Fall	8	9.5	8.6	10.3	-25.0	-25.6	-24.4
HETH	Spring	15	5.6	5.0	6.3	-24.0	-24.5	-23.6
	Fall	10	6.7	5.9	7.5	-24.4	-24.9	-23.9
HOWR*	Spring	9	7.7	6.9	8.5	-23.9	-24.4	-23.3
	Fall	11	10.3	9.5	11.0	-24.8	-25.3	-24.3
LEFL*	Spring	15	7.2	6.6	7.8	-22.5	-23.0	-22.1
	Fall	10	8.9	8.1	9.6	-24.0	-24.5	-23.4
MAWA	Spring	5	5.3	4.2	6.4	-24.0	-24.7	-23.2
	Fall	10	3.5	2.7	4.3	-25.1	-25.6	-24.6
YRWA	Spring	15	5.4	4.7	6.0	-24.1	-24.5	-23.7
	Fall	10	5.4	4.6	6.1	-24.4	-24.9	-23.9
OCWA	Spring	10	5.3	4.5	6.1	-23.4	-24.0	-22.9
	Fall	10	4.5	3.7	5.3	-24.5	-25.1	-24.0
SOSP	Spring	10	8.9	8.2	9.7	-22.2	-22.7	-21.6
	Fall	11	8.5	7.7	9.2	-24.7	-25.2	-24.1
TRES*[a]	Spring	10	9.7	8.9	10.5	-23.5	-24.1	-23.0
	Fall	10	8.2	7.4	8.9	-24.6	-25.1	-24.1
WAVI*	Spring	10	6.0	5.2	6.8	-23.7	-24.2	-23.2
	Fall	10	10.3	9.5	11.1	-24.6	-25.1	-24.0

* Indicates species with significant seasonal differences in $\delta^{15}N$ values based on overlapping confidence intervals.

[a] Sampled over the course of 2 days

Table 2.5 (con't)

Species	Season	n	$\delta^{15}N$ (‰)	95% CI		$\delta^{13}C$ (‰)	95% CI	
Claws				Lower	Upper		Lower	Upper
AMRE	Spring	9	6.5	5.6	7.4	-21.3	-22.0	-20.6
	Fall	10	6.0	5.2	6.9	-22.7	-23.4	-22.1
AMRO*	Spring	13	8.1	7.3	8.8	-20.8	-21.4	-20.3
	Fall	3	10.9	9.3	12.5	-23.1	-24.2	-21.9
BAOR*	Spring	11	7.2	6.4	8.0	-21.6	-22.2	-20.9
	Fall	6	10.3	9.1	11.4	-23.1	-24.0	-22.3
BAWW	Spring	5	5.5	4.3	6.7	-22.0	-22.9	-21.0
	Fall	11	4.7	3.8	5.5	-23.2	-23.9	-22.6
CEDW	Spring	12	6.3	5.6	7.1	-22.9	-23.5	-22.3
	Fall	3	8.6	7.1	10.2	-22.4	-23.5	-21.2
COYE	Spring	17	7.1	6.4	7.7	-22.1	-22.6	-21.6
	Fall	10	8.4	7.5	9.2	-23.2	-23.9	-22.6
GRCA*	Spring	10	6.2	5.3	7.0	-23.1	-23.8	-22.5
	Fall	10	8.9	8.1	9.8	-23.1	-23.8	-22.5
HETH	Spring	15	5.0	4.3	5.7	-22.0	-22.6	-21.5
	Fall	10	5.4	4.5	6.3	-22.8	-23.5	-22.2
HOWR	Spring	9	7.9	7.0	8.8	-20.6	-21.3	-19.9
	Fall	11	9.5	8.6	10.3	-22.8	-23.4	-22.1
LEFL*	Spring	17	6.0	5.4	6.7	-20.7	-21.2	-20.2
	Fall	10	8.0	7.1	8.8	-22.6	-23.2	-21.9
MAWA	Spring	10	4.6	3.7	5.4	-22.1	-22.7	-21.4
	Fall	10	3.1	2.3	4.0	-23.3	-24.0	-22.7
YRWA*	Spring	15	6.4	5.7	7.1	-22.3	-22.9	-21.8
	Fall	10	4.7	3.9	5.6	-22.7	-23.3	-22.0
OCWA*	Spring	12	5.9	5.1	6.7	-22.1	-22.7	-21.5
	Fall	10	3.8	2.9	4.7	-22.6	-23.3	-22.0
SOSP	Spring	9	6.7	5.8	7.5	-18.9	-19.6	-18.3
	Fall	10	7.9	7.0	8.8	-23.0	-23.6	-22.3
TRES[a]	Spring	10	7.7	6.8	8.6	-20.7	-21.4	-20.0
	Fall	10	6.2	5.4	7.1	-23.6	-24.2	-23.0
WAVI*	Spring	10	5.3	4.5	6.2	-21.9	-22.6	-21.3
	Fall	10	9.7	8.9	10.6	-23.1	-23.7	-22.4

* Indicates species with significant seasonal differences in $\delta^{15}N$ values based on overlapping confidence intervals.

[a] Sampled over the course of 2 days

$\delta^{15}N$ values from spring to fall migration, contrary to my prediction, with the exception of Tree Swallows which had higher $\delta^{15}N$ values during spring migration. The remaining omnivores (American Robin, Cedar Waxwing, Hermit Thrush, Yellow-rumped Warbler, Orange-crowned Warbler, Song Sparrow) did not show a significant shift in $\delta^{15}N$ values. There were similar trends for claw $\delta^{15}N$ values. Among the insectivores, only House Wrens and Magnolia Warblers showed a seasonal shift in $\delta^{15}N$ values although differences were marginally non-significant for both species (Table 2.5). These species differed in the direction of their shift; House Wren showed an increase in $\delta^{15}N$ values from winter to summer while Magnolia Warbler had lower $\delta^{15}N$ values during summer compared to winter. Omnivores grouped into three categories based on the direction of seasonal shifts for claw $\delta^{15}N$ values. The first group of omnivores (American Robin, Baltimore Oriole, Gray Catbird, Least Flycatcher, Warbling Vireo) had low $\delta^{15}N$ values during late winter and higher $\delta^{15}N$ values during late summer. The second group (Yellow-rumped Warbler, Orange-crowned Warbler) had higher $\delta^{15}N$ values during late winter compared to late summer. The third group (Cedar Waxwing, Hermit Thrush, Song Sparrow, Tree Swallow) showed no significant difference in $\delta^{15}N$ values between seasons.

For $\delta^{13}C$ values, there was a similar trend for both blood and claws. Generally, $\delta^{13}C$ values decreased from spring migration to fall migration (i.e., based on blood) and from late winter to late summer (i.e., based on claws) (Table 2.5). None of the insectivorous species showed significant seasonal differences in blood $\delta^{13}C$ values. Omnivorous species that had significant seasonal shifts in blood $\delta^{13}C$ values included Least Flycatcher, Orange-crowned Warbler, Song Sparrow, Tree Swallow, and Warbling Vireo. For claw $\delta^{13}C$ values, species that showed a significant difference between late winter and late summer periods included American Redstart and House Wren for insectivores, and American Robin, Baltimore Oriole, Least Flycatcher, Song sparrow, and Tree Swallow for omnivores. These trends followed the prediction that claw $\delta^{13}C$ values would decrease with increasing latitude. The shift from high blood $\delta^{13}C$ values during spring migration to low $\delta^{13}C$ values during fall migration is also consistent if birds fed at a lower trophic level during fall migration. However, species had higher claw $\delta^{13}C$ values during the winter period compared to the breeding season, contrary to

my predictions. The shift from high claw $\delta^{13}C$ values during spring migration to low $\delta^{13}C$ values during fall migration was consistent with birds feeding at a lower trophic level during fall migration.

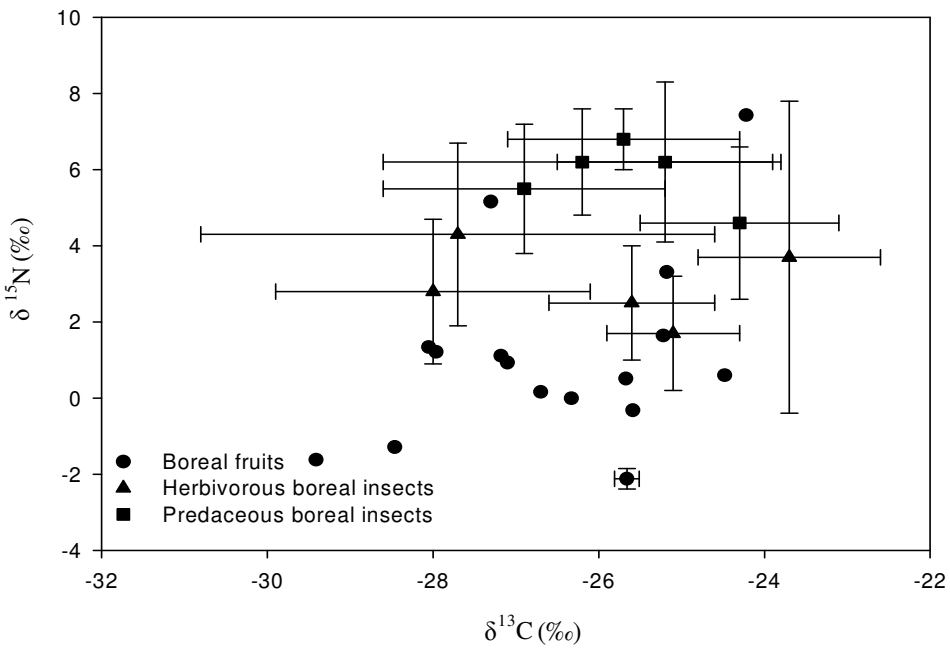
2.3.3. Isotopic Comparison of Food Sources

Boreal predaceous insects and boreal fruits had the highest degree of separation and high within-group homogeneity (T=-7.95, A=0.28, P<0.001) similar to the degree of separation between boreal herbivorous insects and boreal fruits (T=-5.31, A=0.16, P=0.001). There was a comparably low degree of separation and low within-group similarity between agricultural fruits and boreal fruits (T=-2.79, A=0.06, P=0.023), agriculture fruits and boreal herbivorous insects (T=-2.32, A=0.078, P=0.034), and agriculture fruits and boreal predaceous insects (T=-3.32, A=0.11, P=0.012). Boreal herbivorous and predaceous insects had an intermediate degree of separation and the highest within-group homogeneity (T=-4.62, A=0.37, P=0.003) compared to all other group comparisons. All two-way comparisons were highly significant (Figure 2.2).

2.3.4. Age and Sex Class Comparisons

There were no differences in blood and claw $\delta^{13}C$ and $\delta^{15}N$ values between age classes for birds caught in both spring and fall sampling periods (t-test, P>0.08 in all cases), with the exception of Yellow-rumped Warblers caught during spring migration (*Blood* $\delta^{15}N$, t = 2.35, df = 8, P = 0.05; *Blood* $\delta^{13}C$, t = 3.35, df = 8, P = 0.01) where second-year birds had higher blood $\delta^{13}C$ and $\delta^{15}N$ values than after-second-year birds and fall migration (*Blood* $\delta^{15}N$, t = 3.05, df = 8, P = 0.02) where hatch-year birds had higher blood $\delta^{15}N$ values than after-hatch-year birds. As well, there was no difference in $\delta^{13}C$ and $\delta^{15}N$ values between sex classes for both tissues and sampling seasons (t-test, P>0.06 in all cases), with the exception of Orange-crowned Warblers caught during spring migration (*Claw* $\delta^{15}N$, t = -2.45, df = 10, P = 0.04) where females had higher claw $\delta^{15}N$ values than males.

A)



B)

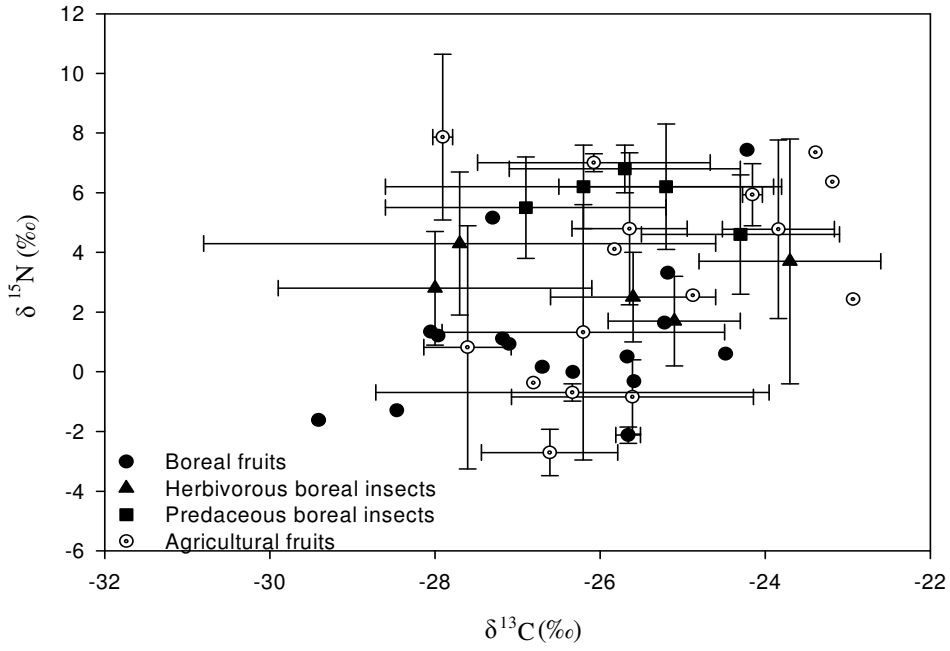


Figure 2.2 Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ of (A) boreal fruits, herbivorous insects, and predaceous insects collected from Prince Albert National Park, SK between June and August 2003. Panel (B) illustrates the same boreal food sources as in panel A with the addition of agricultural fruits collected from Delta Marsh, MB, Redberry Lake, SK and Saskatoon, SK between July and September of 2003.

2.3.5. Capture Day and Body Condition

Blood and claw $\delta^{15}N$ and $\delta^{13}C$ values were regressed against capture day each season. A significant proportion of the variation in isotope values was explained for seven species during spring migration (*Blood* $\delta^{15}N$ – Baltimore Orioles: $F_{1,8} = 7.69$, $r^2 = 0.49$, $P = 0.02$, Common Yellowthroats: $F_{1,10} = 7.96$, $r^2 = 0.44$, $P = 0.02$; *Blood* $\delta^{13}C$ – Tree Swallows: $F_{1,8} = 6.01$, $r^2 = 0.43$, $P = 0.04$; *Claw* $\delta^{15}N$ – Black-and-white Warblers: $F_{1,3} = 17.78$, $r^2 = 0.86$, $P = 0.02$, House Wrens: $F_{1,7} = 9.13$, $r^2 = 0.57$, $P = 0.02$; *Claw* $\delta^{13}C$ – Least Flycatchers: $F_{1,15} = 9.22$, $r^2 = 0.38$, $P = 0.008$, Orange-crowned Warblers: $F_{1,10} = 5.30$, $r^2 = 0.35$, $P = 0.04$) and three species during fall migration (*Blood* $\delta^{15}N$ – Gray Catbirds: $F_{1,6} = 12.60$, $r^2 = 0.68$, $P = 0.01$, Yellow-rumped Warblers: $F_{1,8} = 14.14$, $r^2 = 0.64$, $P = 0.006$; *Blood* $\delta^{13}C$ – Least Flycatchers: $F_{1,8} = 10.96$, $r^2 = 0.58$, $P = 0.01$; *Claw* $\delta^{15}N$ – Gray Catbirds: $F_{1,8} = 14.26$, $r^2 = 0.64$, $P = 0.005$; *Claw* $\delta^{13}C$ – Least Flycatchers: $F_{1,8} = 15.32$, $r^2 = 0.66$, $P = 0.004$). During spring migration, late-arriving birds had higher blood $\delta^{15}N$ values but lower blood $\delta^{13}C$ values. The reverse trend was observed for isotope values of claws with later-arriving birds having lower $\delta^{15}N$ values and higher $\delta^{13}C$ values. During fall migration, the same trend was observed for both blood and claw isotope values; later-arriving birds had lower $\delta^{15}N$ values and higher $\delta^{13}C$ values. It should be noted that Tree Swallows were caught over the course of two days only therefore the significant results for blood $\delta^{13}C$ values are probably not meaningful in terms of evaluating how diet influenced arrival time.

For two species, capture day explained a considerable amount of variation in $\delta^{15}N$ and $\delta^{13}C$ values but these relationships were non-significant (Spring migration: *Blood* $\delta^{13}C$ – Black-and-white Warblers: $F_{1,2} = 5.15$, $r^2 = 0.72$, $P = 0.2$, Magnolia Warblers: $F_{1,3} = 3.39$, $r^2 = 0.53$, $P = 0.2$; *Claw* $\delta^{13}C$ – Black-and-white Warblers: $F_{1,3} = 5.97$, $r^2 = 0.67$, $P = 0.09$).

Body condition indices explained a significant proportion of the variation in blood and claw $\delta^{13}C$ and $\delta^{15}N$ values for three species during spring migration (*Blood* $\delta^{15}N$ – Black-and-white Warblers: $F_{1,2} = 18.6$, $r^2 = 0.90$, $P = 0.05$; *Claw* $\delta^{13}C$ – American Redstarts: $F_{1,6} = 5.94$, $r^2 = 0.5$, $P = 0.05$, Gray Catbirds: $F_{1,8} = 7.5$, $r^2 = 0.48$, $P = 0.03$) and two species during fall migration (*Blood* $\delta^{15}N$ – American Redstarts: $F_{1,7} = 8.76$, $r^2 = 0.56$, $P = 0.02$, Baltimore Orioles: $F_{1,3} = 22.02$, $r^2 = 0.88$, $P = 0.02$; *Blood* $\delta^{13}C$ – American Redstarts: $F_{1,7} = 5.97$, $r^2 = 0.46$, $P = 0.05$; *Claw* $\delta^{15}N$ –

American Redstarts: $F_{1,7} = 12.48$, $r^2 = 0.64$, $P = 0.01$; *Claw* $\delta^{13}C$ – American Redstarts: $F_{1,7} = 8.85$, $r^2 = 0.56$, $P = 0.02$, Baltimore Orioles: $F_{1,4} = 18.29$, $r^2 = 0.82$, $P = 0.01$). During spring migration, birds with lower blood and claw $\delta^{13}C$ and $\delta^{15}N$ values had a higher body condition value. During fall migration, birds with higher body condition had higher blood and claw $\delta^{15}N$ values and lower blood and claw $\delta^{13}C$ values, with the exception of Baltimore Orioles which had higher claw $\delta^{13}C$ values.

2.4. DISCUSSION

Substantial variation in $\delta^{13}C$ and $\delta^{15}N$ values was found in blood and claws of songbirds migrating through the stopover site in southern Manitoba. However, contrary to expectation, there was no clear isotopic segregation between birds I considered to be insectivorous and those considered omnivorous based on their $\delta^{15}N$ values. Although my predictions concerning seasonal patterns in $\delta^{15}N$ values were generally supported, my results indicate that the use of stable isotope analyses to evaluate the incidence of frugivory is generally more complex than I anticipated.

2.4.1. Diet Guilds

Frugivory in songbirds has been documented to varying degrees during spring and fall migration periods and winter (reviewed by Parrish 2000). Thus, among omnivorous species, I expected to detect a range in tissue $\delta^{15}N$ values and, indeed, this pattern was observed. Furthermore, I expected less isotopic variance among species during the breeding season since diets are dominated by insects at that time (Bairlein 1990, Berthold 2001). However, fall claw $\delta^{15}N$ values varied widely across species, possibly an indication of birds feeding on insects from different trophic levels or the occurrence of birds using a variety of food webs differing in baseline $\delta^{15}N$ values. Less variation in tissue $\delta^{13}C$ values reflected either the more limited trophic effect for that isotope and/or the more isotopically homogenous $\delta^{13}C$ landscapes experienced by these birds.

Segregation between insectivores and omnivores based on tissue $\delta^{15}N$ values did not occur as predicted. Overall, insectivores did not have higher $\delta^{15}N$ values compared to omnivorous species and there was a substantial amount of overlap in $\delta^{15}N$ values

between the two guilds. Unexpectedly, American Redstarts, Black-and-white Warblers, and Magnolia Warblers had low blood and claw $\delta^{15}N$ values in both sampling seasons relative to other insectivores and the majority of omnivores suggesting that these insectivorous birds may have been feeding on fruits. Alternatively, these species may be wintering in habitats with low food web $\delta^{15}N$ values but there are few studies that have evaluated $\delta^{15}N$ values in food samples on the wintering grounds of these species. Previous studies have provided little or no evidence that these species feed on fruits (Blake and Loiselle 1992, Hall 1994, Kricher 1995, Parrish 1997, Sherry and Holmes 1997). Blake and Loiselle (1992) found no fruit material in fecal samples of Magnolia Warblers and Black-and-white Warblers; however, this was based on very low sample sizes. Tramer and Tramer (1977) observed Magnolia Warblers feeding on *Lonicera* sp. berries during migration but only in inclement weather. Parrish (1997) detected small amounts of fruit material in fecal samples from Black-and-white Warblers and American Redstarts during fall migration but they remained classified as insectivores. On the other hand, Magnolia Warblers were reclassified as omnivores because fecal samples contained a sufficient percentage of fruits (Parrish 1997). At the other end of the spectrum, American Robins consistently had the highest blood and claw $\delta^{15}N$ values compared to other species. I had predicted that robins would have some of the lowest $\delta^{15}N$ values because it has been well documented that this species feeds on substantial amounts of fruits during their annual cycle (Martin et al. 1951, Wheelwright 1986, White and Stiles 1990). Aside from fruits, earthworms are an important part of their diet during spring and summer (Sallabanks and James 1999). In European soils, Briones et al. (2001) showed that earthworms had high $\delta^{15}N$ values (8-10 ‰). If this also holds true for earthworms found in North American soils, then this could explain the high blood and claw $\delta^{15}N$ values observed in robins. Cedar Waxwings are considered to be one of the most frugivorous songbirds in North America with fruit constituting up to 84% of their annual diet (Witmer et al. 1997). Nevertheless, blood and claw $\delta^{15}N$ values from Cedar Waxwings were higher than several species considered insectivorous and omnivorous.

Within-species, seasonal patterns in isotopic signatures demonstrated that a large proportion of both insectivores and omnivores did not follow my predictions. Among

insectivores, American Redstarts, Black-and-white Warblers, and Common Yellowthroats showed no seasonal changes in blood and claw $\delta^{15}N$ values as expected. However, House Wrens and Magnolia Warblers had seasonal differences in blood and claw $\delta^{15}N$ values, although results were marginally non-significant. Johnson (1998) stated that House Wrens were primarily insectivorous and provided no evidence of fruits in their diet. Most previous information on diet of House Wrens was gathered during breeding periods (e.g., Guinan and Sealy 1987), a time when an insect-based diet would be expected. To my knowledge, there are no studies that have examined the diet of this species during migration and winter. One of the main food sources for House Wrens is spiders which can have relatively high $\delta^{15}N$ values due to their predatory habits (Table 2.3). Therefore, the seasonal differences in blood and claw $\delta^{15}N$ values observed in House Wrens may be attributable to a shift between high and low trophic level insects. There is limited information about the year-round diet of Magnolia Warblers. However, some studies have documented that this species eats fruits at different times of the year (Tramer and Tramer 1977, Hall 1994, Parrish 1997, Parrish 2000 and references within).

Diet shifts were observed in omnivorous species. In accordance with my predictions, a significant shift in blood $\delta^{15}N$ values only occurred for Tree Swallows while seasonal changes in claw $\delta^{15}N$ values were observed for American Robins, Baltimore Orioles, Gray Catbirds, Least Flycatchers, and Warbling Vireos. The majority of species either showed no change in tissue $\delta^{15}N$ values between seasons or a seasonal shift in $\delta^{15}N$ values in the opposite direction to my predictions. No seasonal shift in $\delta^{15}N$ values were observed during migration periods for American Robins, Cedar Waxwings, Hermit Thrushes, and Yellow-rumped Warblers which suggests similar diets during spring and fall migration. This contrasts with previous studies documenting a heavy use of fruits by American Robins, Hermit Thrushes, and Yellow-rumped Warblers during fall migration (Blake and Hoppes 1986, Malmborg and Willson 1988, Parrish 1997, Suthers et al. 2000) and to a lesser extent during spring migration (Wheelwright 1986, Jones and Donovan 1996, Hunt and Flaspohler 1998, Strong et al. 2005). Cedar Waxwings feed on berries more consistently throughout the year (Witmer 1996); therefore, it is perhaps less surprising that there was no significant change in $\delta^{15}N$ values between periods for this species. Hermit Thrushes also showed no significant difference

in $\delta^{15}N$ values between winter and summer which was unexpected because it has been well documented that berries are a main component of their winter diet (Martin et al. 1951, Jones and Donovan 1996, Kwit et al. 2004a, Strong et al. 2005). Furthermore, Yellow-rumped Warblers feed almost exclusively on southern bayberry (*Myrica cerifera*), or other fruits when bayberries are unavailable during winter (Hausman 1927, Hunt and Flaspohler 1998 and references therein, Kwit et al. 2004a), therefore higher claw $\delta^{15}N$ values in winter compared to summer were unexpected in this case.

My results indicated higher claw $\delta^{13}C$ values during winter compared to breeding season. Values of $\delta^{13}C$ in food webs can increase with decreasing latitude. Also, higher trophic-level feeding in winter would also contribute to this effect. It is difficult to distinguish between the magnitude of a latitudinal effect and the trophic effect in $\delta^{13}C$ values until dietary $\delta^{13}C$ values are available from the wintering grounds. For example, if birds were feeding on more fruits during winter, I would expect lower $\delta^{13}C$ values but latitudinal effects could work in the other direction (i.e., increasing $\delta^{13}C$ values) and so possibly masking any changes in ^{13}C attributable to diet changes.

Guilds were subjective due largely to a lack of information from the literature for most species. Indeed, studies have documented the occurrence of fruit in fecal samples or stomach contents from species of migratory songbirds that were once thought to be exclusive insectivores (e.g., Parrish 1997). Even though the presence of fruits may have been minimal or observed in few individuals, the degree to which these species included fruits in their diet remains unknown. As Suthers et al. (2000) pointed out, it is clear that guild classifications of most migratory songbirds must be revised because most species are known to eat fruit. Furthermore, guild classifications should be assigned by season to more accurately reflect dietary variations that occur throughout a bird's annual cycle. As such, more study is needed to gain a more complete picture of diet during winter and migration periods of migratory songbirds.

Potential explanations for the lack of fit between my isotopic results and species dietary expectations include the possibility that the actual incidence of frugivory for these species is simply misleading or that the basis of the use of stable isotope analysis to answer such questions, namely that insects are always enriched in ^{15}N compared to fruits in all landscapes, is invalid.

2.4.2. Frugivory in Migratory Passerines

The need to replenish energy reserves while *en route* promotes flexibility in the diets of migrant songbirds (Parrish 2000), an important adaptation because birds will use several stopover sites varying in habitat and food resources. Indeed, migrants are likely to feed on any available food resources at stopover sites that will aid in replenishing their energy stores (Bairlein and Gwinner 1994) and these food sources likely vary from location to location and from year to year (Malmborg and Willson 1988).

Songbirds may incorporate fruits into their diets when insects become scarce (Bairlein 1990) regardless of which diet guild they may be associated with (Parrish 1997). Also, there is some evidence that species simply shift to different insect types or fruit species (e.g., Bibby and Green 1981) instead of changing trophic level per se. Perhaps this was the case with species that did not show a significant shift in tissue $\delta^{15}N$ values but were expected to do so.

The DMBO is located near (approximately 174 km from the southern edge of the low boreal forest, Google Earth 2006) the breeding grounds of birds nesting in Manitoba's low boreal forest. Therefore these birds were probably at the beginning of their migration period. As such, it is possible that certain species had not changed their diet yet because insects were still available and/or preferred at that time. However, there was evidence of frugivory from berry stains on and around the bill and cloaca of American Robins, Gray Catbirds, Song Sparrows, and Hermit Thrushes caught at the Delta Marsh Bird Observatory during fall migration (M. C. Gagnon, pers. obs.), the same species that did not show any change in blood and claw $\delta^{15}N$ values between seasons (with the exception of Gray Catbirds and claw $\delta^{15}N$ values of American Robins). Therefore, tissue isotopic values may not have provided the expected delineation between fruits and insects or recent foraging on berries may not have been reflected in tissue $\delta^{15}N$ values yet.

2.4.3. Isotopic Signature of Food Webs

Environmental factors that can affect soil and plant $\delta^{15}N$ values include climate, topography, soil age, soil parent material, and human activity (Vitousek et al. 1997,

Amundson et al. 2003). Human activity has caused alterations in the nitrogen content and nitrogen isotopic composition of soils and plants (Vitousek et al. 1997). Ultimately, this alters the isotopic composition of food webs in landscapes that are affected by anthropogenic activities and complicates the interpretation of $\delta^{15}N$ values in trophic-level studies at landscape or continental scales. Although the fruits and insects I sampled in the boreal forest were isotopically distinct and showed the expected shift by trophic level, $\delta^{15}N$ values of fruits sampled in agricultural areas were highly variable, generally had higher $\delta^{15}N$ values and overlapped both insect and fruit sources from the boreal forest. I did not sample insects from agricultural areas and therefore cannot comment on the isotopic signatures of this food source. However, Hobson (1999b) demonstrated an enrichment in ^{15}N from insects and bird tissues in agro-wetland complexes (including Delta Marsh) compared to those from the boreal forest (Prince Albert National Park). Disturbed ecosystems, such as cultivated fields, have been characterized by high levels of biological activity, accelerating the loss of soil ^{14}N through microbial nitrogen metabolism and subsequently causing the remaining soil to become enriched in ^{15}N relative to undisturbed land (Lajtha and Michener 1994, Amundson et al. 2003). Additionally, the use of fertilizer for agricultural purposes also contributes to higher $\delta^{15}N$ values in cultivated soils through ammonification (Vitousek et al. 1997, Amundson et al. 2003). When comparing agricultural and forest soils, Riga et al. (1971) demonstrated up to a 3‰ increase in $\delta^{15}N$ values to a depth of 60 cm due to cultivation; they also showed an increase in soil $\delta^{15}N$ values in agricultural fields when treated with fertilizer relative to untreated agricultural fields.

The discrepancy in $\delta^{15}N$ values between forest and agriculture landscapes could help explain some of the unusual isotopic patterns I encountered. For example, American Robins are often associated with urban and agricultural areas and it has been well documented that they feed on substantial amounts of fruit during fall migration and winter in these habitats (Sallabanks and James 1999). Similarly, Cedar Waxwings are also associated with urban and agricultural areas where ornamental trees and shrubs provide them with crops of fruit (Witmer et al. 1997). Therefore, the high $\delta^{15}N$ values detected in blood and claws of these species may have reflected food web enrichment in ^{15}N due to anthropogenic activities in agricultural or settled areas. Furthermore, isotopic

differences between these habitats may help explain the seasonal change in tissue $\delta^{15}N$ values observed in certain species that was opposite to my predictions. Baltimore Orioles, Gray Catbirds, Least Flycatchers, and Warbling Vireos had higher blood $\delta^{15}N$ values in the spring compared to fall and Yellow-rumped Warblers and Orange-crowned Warblers had higher claw $\delta^{15}N$ values in the winter compared to summer. Feeding in agricultural areas in winter and/or at various stopovers during spring migration may be responsible for the enriched tissue ^{15}N. There is extensive evidence in the literature that Yellow-rumped Warblers feed mainly on berries during winter (Hausman 1927, Martin et al. 1951, Hunt and Flaspohler 1998) therefore it seems unlikely that the higher $\delta^{15}N$ values reflect an insect-dominated diet at that time. A plausible explanation would be that agricultural activities have caused food webs in these landscapes to become enriched in ^{15}N; consequently, 'artificially' high $\delta^{15}N$ values are reflected in tissues of consumers. Yet, stable isotope analysis of southern bayberries from Florida revealed $\delta^{15}N$ values comparable to my boreal fruits (Pearson, S.F. and Levey, D.J., pers. comm., mean (SD) = -2.3 (0.3) ‰ for $\delta^{15}N$ and -30.0 (1.2) ‰ for $\delta^{13}C$). Therefore it appears that the isotopic delineation of fruits may work for some sites but not uniformly across North America.

Climate can influence soil and plant $\delta^{15}N$ values. Amundson et al. (2003) demonstrated, on a global scale, that soil and plant $\delta^{15}N$ values decrease as mean annual precipitation increases and mean annual temperature decreases. As such, these patterns are reflected by lower soil $\delta^{15}N$ values occurring in northern latitude temperate ecosystems and higher soil $\delta^{15}N$ values occurring in arid and tropical environments (Martinelli et al. 1999, Amundson et al. 2003). Therefore, plants in southern U.S., Mexico, and Central America, which represent wintering areas for many of my study species, may be naturally enriched in ^{15}N compared to temperate and boreal ecosystem plants. This may have contributed to unexpected seasonal patterns in avian tissue $\delta^{15}N$ values.

Between boreal and agricultural zones, there was variation in isotopic signatures among different species of fruits or insect Orders. Fruit $\delta^{15}N$ values varied between species at the same site, between individuals of the same species at the same sites (Table 2.2), and between the same species at different sites. Handley et al. (1999) showed that

plant $\delta^{15}N$ values can vary between different species of plants at the same site. $\delta^{15}N$ values in plant tissues can vary depending on how plants fix nitrogen. Nitrogen-fixing plants derive their nitrogen directly from the atmosphere while non-N-fixing plants obtain nitrogen from the soil (Lajtha and Marshall 1994). Since soil nitrogen is more enriched in ^{15}N compared to atmospheric nitrogen (Shearer et al. 1978), non-N-fixing plants are expected to have higher $\delta^{15}N$ values than N-fixing plants. Most of the plants sampled in my study are non-N-fixers with the possible exception of species belonging to the *Rubus* and *Shepherdia* genera (Torrey et al. 1978). However, $\delta^{15}N$ values from *Shepherdia canadensis*, *S. argentea*, and *Rubus idaeus* were similar to those of non-N-fixing plants. Clearly, there were site-specific factors that affected the isotopic composition of flora causing substantial intra- and interspecific variability.

I based my hypotheses about expected seasonal diet shifts on a simple isotopic trophic-enrichment model between plants and insects. However, insects occupy different trophic levels (Romoser and Stoffolano 1998). Park and Lee (2006) examined arthropod trophic relationships in a temperate ecosystem using $\delta^{13}C$ and $\delta^{15}N$ measurements and demonstrated a consistent increase in $\delta^{15}N$ values in the order of soil, plants, herbivores, predators/parasitoids, and detrivores. In their study, $\delta^{15}N$ enrichment from plants to herbivorous insects was reported at only 0.3‰. Spence and Rosenheim (2005) documented a mean $\delta^{15}N$ difference between plants and herbivorous insects of 1.9 ± 0.4‰, although there was substantial variation associated with this value depending on different herbivore-host plant associations. These studies demonstrate that the isotopic difference between plants and herbivorous insects may be small and far less than the average 3.4‰ found across foodwebs (Vanderklift and Ponsard 2003). The absence of a large $\delta^{15}N$ difference between insects and fruits may have prevented us from detecting frugivory in species like American Robin, Hermit Thrush, and Song Sparrow. In tropical systems, previous researchers like Herrera et al. (2001a, 2001b, 2003) have found large differences between insects and fruits (~3 ‰). Furthermore, insectivorous species that had unexpectedly low $\delta^{15}N$ values (American Redstarts, Black-and-white Warblers, and Magnolia Warblers) may have fed predominantly on low trophic-level insects (e.g., leaf beetles, click beetles, Lepidoptera spp.).

2.4.4. Body condition and capture day

It has been suggested that switching from an insect diet to a fruit-dominated diet, especially during fall migration, occurs to assist in the accumulation of fat which is the main energy source used to fuel migratory flights (Martin et al. 1951, Bairlein and Gwinner 1994, Berthold 2001). Nutritionally, insects are generally high in protein and lipid content but variable in carbohydrates; in contrast, fruits are usually low in protein, high in carbohydrates, and have a variable lipid content (Johnson et al. 1985, Moermond and Denslow 1985). Birds feeding on low-lipid, carbohydrate-rich fruits, which are most prevalent in temperate North America, show an increase in the assimilation efficiency of lipids allowing them to maximize their intake of this nutrient (Bairlein 1987, Witmer and Van Soest 1998, Lepczyk et al. 2000). Although some studies have shown that birds cannot maintain body mass feeding exclusively on fruits (Berthold 1976, Levey and Karasov 1989, Witmer 1998), there is evidence indicating that frugivory is beneficial to birds in terms of gaining body mass, thereby improving body condition, prior to migration or *en route* (Bairlein 1991, 1994, 1996, 1998, 2002, Parrish 1997, Witmer and Van Soest 1998). Garden Warblers that were fed a mixed diet of both insects and fruits, on which they were able to gain body mass, had a significantly higher daily rate of mass gain then birds feeding only on insects (Bairlein 1996). Furthermore, Parrish (1997) demonstrated that migrants that had more than one-third of their diet as fruits during stopover had better body condition and were more successful at gaining body mass than insectivores prior to departure.

As such, I expected omnivorous species feeding on both fruits and insects to have greater body condition. However, this was not the case. The only omnivores for which body condition had a significant influence on isotope values were Baltimore Orioles and Gray Catbirds. American Redstarts and Black-and-white Warblers were the only insectivorous species for which body condition indices had a significant influence on $\delta^{15}N$ and $\delta^{13}C$ values. As illustrated by factors discussed previously, the ambiguity in $\delta^{15}N$ values accurately representing fruit and insect diets at low and high trophic levels, respectively, limits my ability to make conclusions regarding the effect diet has on body condition of these migrating songbirds. For example, in the case of American Redstarts sampled during fall migration, $\delta^{15}N$ values ranged from 4.5 ‰ to 9.2 ‰ and 4.6 ‰ to

8.2 ‰ for blood and claws, respectively. Therefore, it is not known whether these data were indicative of a diet change between fruits and insects or between herbivorous and predaceous insects (e.g. switching from butterflies (Lepidoptera, $\delta^{15}N = 2.8$ ‰) to spiders (Araneae, $\delta^{15}N = 6.8$ ‰)).

The only species with a significant relationship between $\delta^{15}N$ and $\delta^{13}C$ values and arrival time were Baltimore Oriole, Common Yellowthroat, Tree Swallow, Black-and-white Warbler, House Wren, Least Flycatcher and Orange-crowned Warbler captured in the spring and Gray Catbird, Yellow-rumped Warbler, and American Robin captured in the fall. To my knowledge, no studies have examined how a bird's dependence on a particular food source (i.e., fruits and/or insects) corresponds to its timing of arrival, however Marra et al. (1998) did find evidence that habitat differences (i.e., indexed by $\delta^{13}C$ values) contributed to arrival time differences. The relationships I observed between blood and claw $\delta^{15}N$ and $\delta^{13}C$ values and capture day cannot be easily interpreted. Given the opposing directions of $\delta^{15}N$ and $\delta^{13}C$ values observed from both sampling seasons, it seems that $\delta^{13}C$ values are more likely to represent changes in feeding environments instead of small changes in trophic level. For example, keeping in mind that plant $\delta^{13}C$ values are higher in dry areas compared to wet areas (Lajtha and Marshall 1994), fall data suggested that later-arriving birds were feeding on more fruits in dry areas (i.e., low $\delta^{15}N$, high $\delta^{13}C$) while insects from wet areas (i.e., high $\delta^{15}N$, low $\delta^{13}C$) dominated the diet of earlier-arriving birds. Spring data for blood suggested that birds arriving later were feeding on insects from wet areas (i.e., high $\delta^{15}N$, low $\delta^{13}C$). This would represent their migration diet. Based on spring claw data, later-arriving birds had low $\delta^{15}N$ and high $\delta^{13}C$ values suggesting that, during winter, these birds are feeding on fruit in dry areas.

2.4.5. Evaluation of Stable Isotope Analysis for Studying Frugivory

The usefulness of stable isotope analysis to investigate frugivory in migratory songbirds will depend on the choice of tissues, and their associated turnover rates relative to periods of actual dietary change. The diet integration period for whole blood was estimated at 2-3 weeks in small passerines studied in captivity (Hobson and Bairlein 2003, Pearson et al. 2003). Spring migration tends to occur over a short period of time,

approximately a few weeks, compared to autumn migration which can extend over a period of one to three months, depending on whether a species is a long-, medium-, or short- distance migrant (Alerstam and Lindstrom 1990, Berthold 2001). The DMBO is located near the southern edge of the boreal forest and so, for some birds, $\delta^{15}N$ values from blood collected during fall migration may have reflected a post-breeding/pre-migration diet instead of a migration diet. This could provide a possible explanation for why Gray Catbirds, Least Flycatchers, Baltimore Orioles, House Wrens, and Warbling Vireos, all of which breed in the southern portions of the boreal forest and the aspen parkland (Briskie 1994, Cimprich and Moore 1995, Johnson 1998, Rising and Flood 1998, and Gardali and Ballard 2000, respectively) had higher $\delta^{15}N$ values in fall compared to spring. Claws should provide useful information about premigratory habitats at least four weeks after departure from their previous location (Bearhop et al. 2003). Even though the usefulness of claws depends on wear and growth rates of claws (Bearhop et al. 2003, Mazerolle and Hobson 2005), this tissue remains a valuable option for estimating winter and breeding ground diets, especially in the absence of an alternative tissue. However, as with blood, my ability to pick up the dietary signal of interest depended on when the diet change occurred relative to tissue turnover rates and so using claws may have been relatively crude for this purpose since I had little information about the exact period represented by the distal claw samples I collected.

Proper interpretation of $\delta^{15}N$ and $\delta^{13}C$ values in avian tissues is also dependent on understanding diet-tissue discrimination factors (Hobson and Clark 1992b, Pearson et al. 2003). Dietary alternatives that differ in C and N concentrations will typically involve different diet-tissue discrimination factors (Pearson et al. 2003, Vanderklift and Ponsard 2003). Pearson et al. (2003) determined $\delta^{15}N$ and $\delta^{13}C$ diet-tissue discrimination factors of whole blood and blood plasma from captive Yellow-rumped Warblers and examined the influence of diet C and N concentrations on these discrimination factors. Discrimination factors decreased as the proportion of fruits in the diet increased (i.e. high C:N diet). Alternatively, it has been proposed that diet-tissue discrimination factors for $\delta^{15}N$ will increase with decreasing dietary protein quality causing discrimination factors to decrease with trophic level because carnivores ingest higher quality protein compared to herbivores (Roth and Hobson 2000). In a meta-

analysis, Robbins et al. (2005) showed a decrease in discrimination as protein quality increased with trophic level. However, there was no significant relationship between discrimination factors and dietary nitrogen content (%N, see also Hobson and Bairlein 2003, Vanderklift and Ponsard 2003). Robbins et al. (2005) concluded that discrimination factors across diet groupings were driven by dietary protein quality while the considerable amount of within-group variability in discrimination factors may be related to protein intake relative to animal requirements, thus proposing that both protein quality and quantity may ultimately be important to understand diet-tissue discrimination factors.

Unfortunately, at the moment there is not enough support in the literature to conclusively reject either of these two hypotheses. If diet-tissue discrimination factors are greater between birds and insects than between birds and fruits, there would be no problems interpreting results because the isotopic difference between fruits and insects would simply be accentuated, reinforcing isotopic trophic differences. On the other hand, if discrimination factors are indeed higher in birds feeding on fruits (i.e. a diet with low %N, low protein quality, and high C:N ratio) compared to discrimination factors between birds and insects, as suggested by Roth and Hobson (2000) and Robbins et al. (2005), then the proportion of fruits in a bird's diet could be underestimated as these birds would have high tissue $\delta^{15}N$ values inconsistent with a fruit diet. In essence, this would minimize the initial isotopic difference between fruits and insects.

2.5 CONCLUSION

Based on my findings, evaluating the extent of frugivory in North American migratory songbirds has proven to be a complex task and influenced by multiple factors. The fact that these migrant birds are sampling foodwebs over great distances and different landscapes throughout their annual life cycle, as well as the vast isotopic variability among and between fruits and insects, and the uncertainty about the true discrimination factors for each of these food types makes it advisable not to rely on the stable isotope approach to track frugivory in migrant songbirds.

CHAPTER 3: ANNUAL DIET VARIATIONS OF YELLOW-RUMPED WARBLERS: A STABLE ISOTOPE INVESTIGATION

3.1. INTRODUCTION

Diets of many songbirds are characterized by seasonal variations in the relative proportions of different food sources such as insects and fruits (Wheelwright 1988, White and Stiles 1990, Parrish 1997). This dietary plasticity represents an interesting adaptation and may have played an important role in the evolution of migratory behaviour in some species. For example, there is extensive evidence that migratory songbirds use fruits as a main or supplementary food source during fall (Johnson et al. 1985, White and Stiles 1990, Parrish 1997, Suthers et al. 2000), winter (Blake and Loiselle 1992, Parrish 2000, Prather 2000, Borgmann et al. 2004, Kwit et al. 2004a), and potentially spring (Willson 1991; see references within Parrish 2000) whereas they are almost exclusively insectivorous during the breeding season. The nature of the nutritional and ecological adaptations associated with seasonal frugivory, especially for migrating individuals, has long fascinated biologists and nutritional physiologists (Bairlein and Simons 1995, Witmer 1998, McWilliams et al. 2004). However, studying the diet of songbirds during their annual cycle is difficult, in part due to the challenges of tracking songbirds as they migrate through various landscapes. As well, their use of stopover areas can be inconsistent from year to year (Mehlman et al. 2005) and so it is not easy to pin-point which stopover habitats, and their associated food resources, birds use during migration. So far, no studies have demonstrated when and to what extent diet changes occur throughout the year for most migratory species.

Frugivory in omnivorous songbirds has previously been studied at different times of the year (e.g., Johnson et al. 1985, Blake and Loiselle 1992, Prather 2000). The use of traditional methods (e.g. stomach content analysis, fecal samples) to study frugivory has disadvantages. Different food types are digested at different rates resulting in a potentially biased representation of ingested food items and individuals with empty

stomachs provide no information on types of prey eaten (Gavett and Wakeley 1986, Rosenberg and Cooper 1990). More and more, ecologists have turned to stable isotope analysis when conducting dietary studies. This technique has been very useful for examining trophic relationships between consumers (Hobson 1993, Hobson et al. 2000b), studying temporal changes in diet (Haramis et al. 2001) and determining relative contributions of animal and plant food sources in avian and mammalian vertebrates (Hobson 1993, Kwak and Zeddler 1997, Herrera et al. 2002, Herrera et al. 2005, Urton and Hobson 2005).

The use of stable isotope analysis to examine diets is based in large part on a stepwise enrichment of ^{15}N in consumer tissues that occurs across trophic levels (e.g. herbivory vs. insectivory) with an average enrichment of 3.4 ‰ (Kelly 2000, Post 2002). Therefore, diet shifts may be detected by quantifying $\delta^{15}N$ variations in tissues of consumers (Herrera et al. 2001a, Herrera et al. 2003). A slight increase of approximately 1 ‰ in food web $\delta^{13}C$ values with trophic level has also been noted (Peterson and Fry 1987) although stable carbon isotopes typically provide information on source of feeding (e.g. C_3 vs. C_4 environments, Hobson 2003). Because stable isotope ratios of elements are measured in tissues of consumers, the use of this technique augments traditional methods by providing information on assimilated diet vs. recently ingested foods, over a period of days, months and possibly years depending on choice of tissues. The period for which the isotopic signature of an animal's tissue will reflect the diet's isotopic composition will depend on the tissue's elemental turnover rate. Tissues with fast turnover rates, such as blood plasma and liver, represent diet integrated over periods of 3 to 6 days as determined from Yellow-rumped Warblers for blood plasma and Japanese Quails for liver, while tissues with slower turnover rates, such as muscle and whole blood, reflect diet over a longer period, approximately 2 to 3 weeks as determined from Japanese Quails for muscle and Yellow-rumped Warblers for whole blood (Hobson and Clark 1992a, Pearson et al. 2003, Podlesak et al. 2005). Bone collagen has a very slow turnover rate of several months and therefore likely approximates a lifetime average diet for adult birds (Stenhouse and Baxter 1979, Hobson and Clark 1992a). Feathers are metabolically inactive tissues following formation so they will retain the isotopic signature of the local food web where they were grown.

Therefore, by using multiple tissues, an individual's diet can be reconstructed for various time periods and indicate if any diet shifts have occurred (Tieszen et al. 1983, Hobson and Clark 1992a).

To my knowledge, very few studies have directly examined frugivory in wild North American migratory passerines using stable isotope analysis (see Podlesak et al. 2005). However, this technique has been used to investigate the role of fruits and insects in the diets of tropical passerines (Herrera et al. 2005) and Neotropical frugivorous bats (Herrera et al. 2001a, 2001b). Successful use of stable isotope analysis to determine the extent to which birds depend on fruit and insect resources will increase our ability to establish dietary and habitat requirements of migratory songbirds and thus contribute to the conservation and management of songbird populations and their habitats throughout their annual cycle.

My objectives were to use $\delta^{13}C$ and $\delta^{15}N$ values of multiple tissues from a migratory population of Yellow-rumped Warblers (*Dendroica coronata*) to 1) examine correlations in isotopic signatures among different tissues, 2) evaluate annual diet shifts at the population level, as reflected by variations in $\delta^{13}C$ and $\delta^{15}N$ values, and 3) derive estimates of the proportions of fruit and insects incorporated into their diet during summer and fall using a two-endpoint mixing model. Numerous studies have documented diet shifts in Yellow-rumped Warblers with fruits prevailing in fall migration and winter diets (Hausman 1927, Yarbrough and Johnston 1965, Wilz and Giampa 1978, Malmborg and Willson 1988, White and Stiles 1990, Place and Stiles 1992, Parrish 1997, Suthers et al. 2000, Borgmann et al. 2004, Kwit et al. 2004, Podlesak et al. 2005) and insects in breeding and spring migration diets (Bent 1953, Yarbrough and Johnston 1965, Parrish 2000). To determine over which time periods tissues gave similar diet information, I assumed that well-correlated tissues would represent similar diet. According to previous experimental studies (Hobson and Clark 1992a, Hobson and Bairlein 2003), muscle and whole blood tissues have similar turnover rates and so I predicted that there would be a significant positive correlation in isotope values between these two tissues. Moreover, I predicted that $\delta^{15}N$ values of tissues representing summer diet would be higher than those representing winter and migrations periods, and that tissues representing fall migration would be higher than

those representing spring migration because there is no evidence of fruits in the diet of Yellow-rumped Warblers during spring migration and summer (Hunt and Flaspohler 1998). Little variation in $\delta^{13}C$ values was expected because there is no evidence that Yellow-rumped Warblers feed on C_4 or CAM plants. Furthermore, I predicted that Yellow-rumped Warblers would have a greater proportion of fruits in their diet during fall, compared to summer because fall in North America is the time of year when a substantial amount of fruits have been documented in the diet of Yellow-rumped Warblers (White and Stiles 1990, Parrish 1997, Suthers et al. 2000, Podlesak et al. 2005). Finally, I predicted that Yellow-rumped Warblers would demonstrate a reliance on southern bayberries (*Myrica* spp.) during winter because a strong association between Yellow-rumped Warblers and bayberries has been documented on several occasions and these berries have long been known as its preferred food item during winter (Hausman 1927, Martin et al. 1951, Bent 1953, Wilz and Giampa 1978, Borgmann et al. 2004).

3.2. METHODS

3.2.1. Study Area

During spring (1 – 27 May 2003) and fall (1 August to 30 September 2003) migration of 2003, avian tissue collections were made at the Delta Marsh Bird Observatory (DMBO), located in the dune-ridge forest of Delta Marsh, Manitoba, Canada (98°23' W, 50°11' N) (MacKenzie 1982). This forest, which abuts the south end of Lake Manitoba, is a primary stopover site for migratory songbirds *en route* to and from their breeding and wintering grounds (Mazerolle et al. 2005).

3.2.2. Study Species

The Yellow-rumped Warbler is a short- to medium-distance North American migrant which breeds primarily in the boreal forest and winters in the southeastern United States (Hunt and Flaspohler 1998). This species commonly shifts from an insect diet during summer to a diet dominated by fruits during fall and winter (Hunt and Flaspohler 1998). Also, it is well known for its substantial use of bayberries, a wax-coated fruit found abundantly along the coastal regions of the eastern United States (a common migration route for North American passerines) and on wintering grounds in

the southeastern United States (Yarbrough and Johnston 1965, Wilz and Giampa 1978, Parrish 1997). The Yellow-rumped Warbler's unique ability to digest bayberry wax may allow this species to winter farther north than other wood warblers (Morse 1989, Place and Stiles 1992).

3.2.3. Avian Sampling

Yellow-rumped Warblers were captured during spring and fall migration using standard constant-effort mist netting and banding protocols (Hussell and Ralph 1998). Blood samples were collected from the brachial vein using a sterilized needle (27-gauge) and a heparanized capillary tube (Campbell 1995) then preserved in 70% ethanol (Hobson et al. 1997). Claws were clipped from both the left and right inner and middle toe of all sampled birds using nail clippers. On average, the growth rate of claws in passerines is 0.04 ± 0.01 mm/day (Bearhop et al. 2003). Growth rate of different claws were not expected to vary within the same individual (Bearhop et al. 2003) and so they were selected at random. Approximately 2 to 3 mm of nail from the distal end of the claw was clipped. Yellow-rumped Warblers are not ground foragers therefore it was assumed that the entire part of the claw sampled would adequately represent breeding and wintering ground diets in fall and spring captures respectively (Bearhop et al. 2003, Mazerolle and Hobson 2005). Knowing the moult patterns of Yellow-rumped Warblers, specific feathers that were grown on the breeding and wintering grounds were collected. Prior to spring migration, Yellow-rumped Warblers undergo a pre-alternate moult in which they replace greater coverts (6% of adults and 8% of hatch-year birds may not replace any greater coverts, Pyle 1997). Post-breeding and prior to fall migration, adults have a pre-basic moult in which they replace all body and flight feathers and young birds have a pre-formative moult in which they replace some body feathers (Pyle 1997, Howell et al. 2003). Flight feathers on hatch-year birds are part of the juvenal plumage which was grown at the nest site. As such, greater coverts and tail feathers were collected to represent diet on the wintering grounds and the breeding grounds, respectively. Finally, individuals were aged and sexed using species-specific criteria outlined in Pyle (1997). A subsample of 22 and 40 Yellow-rumped Warblers were collected during spring and fall migration periods, respectively (Animal Care Protocol

#20030018). Liver, muscle and bone were sampled from each bird to have tissues which represent short, medium and long diet integration periods, respectively.

3.2.4. Fruit and Insect Sampling

Food web components were sampled in terrestrial environments to estimate the isotopic range of food sources available to Yellow-rumped Warblers during summer and fall migration the central prairies. Fruits and insects were sampled from southern agricultural areas and the low boreal forest. For the aspen parkland and agricultural regions, ripe fruits were collected between 26 July and 26 September, 2003, at Delta Marsh, Manitoba, and Redberry Lake (107º09' W, 52º43' N), and Saskatoon (106º39' W, 52º07' N), Saskatchewan. Boreal sites included Christopher Lake (105º49' W, 53º33' N) and Emma Lake (105º53' W, 53º35' N), Saskatchewan. We collected 56 samples of fruits representing the genera *Actaea*, *Amelanchier*, *Cornus*, *Lonicera*, *Parthenocissus*, *Prunus*, *Rosa*, *Rubus*, *Sheperdia*, *Solanum*, *Sorbus*, *Symphoricarpos*, and *Viburnum* (see Chapter 2, Table 2.2). Since they are not typically digested by birds, seeds and skin were removed and the fleshy part of each fruit was retained for analysis. Approximately 5 to 60 berries, depending on their size, were combined for each sample to have an adequate amount of material for analysis. We obtained isotopic data for southern bayberry (*Myrica cerifera)* collected during winter in Florida (D. Levey and S, Pearson, pers.comm.).

Insects were not sampled as a part of this study but stable isotope data from a previous study were used (Bennett and Hobson unpublished data). Insects were sampled from May to August 2002 and 2003, in Prince Albert National Park (53°50′ N, 105°50′ W), Saskatchewan and identified to order. For my study, data representing the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Odonata, Orthoptera, and Araneae were used (see Chapter 2, Table 2.3).

3.2.5. Stable Isotope Analysis

Ethanol was decanted from blood samples and the blood then freeze dried. Claws were cleaned of surface oils with a 2:1 chloroform:methanol mixture and allowed to air dry. Lipids were removed from muscle and liver samples using a 2:1

chloroform:methanol mixture, allowed to air dry, and freeze dried. Bone samples were also lipid extracted, allowed to air dry, and ground to a fine powder. Collagen was extracted from each powdered bone sample using the following procedure. Individual powdered bone samples were dissolved in 1M HCl for 20 minutes. This solution was then filtered through a 0.05 μm cellulose membrane filter. Bone collagen retained on the filter paper was collected and placed in a vial containing a few milliliters of distilled water. Finally, collagen samples were freeze dried. Fruit samples were freeze dried and ground to a fine powder using a small mortar and pestle. For consistency, five replicates of each fruit sample were used to generate an average value and eliminate any biases related to potential variance in sample homogeneity.

Approximately 1mg of dry blood, claw, muscle, liver, collagen and insect material and 7mg of fruit material was subsampled for each isotopic measurement. Sample materials were sealed into tin cups, and combusted at 1200ºC in a Robo-Prep elemental analyzer. The resultant CO_2 and N_2 gases were then analyzed using a Carlo Erba elemental analyzer interfaced with a Europa 20:20 continuous-flow isotope-ratio mass spectrometer (CF-IRMS). Laboratory standards of egg albumen and whale baleen were used for blood, claw, muscle, liver, bone collagen and insect samples, and a peagrain standard was used for fruit samples.

Samples were analyzed at the Soil Science Laboratory, University of Saskatchewan, and the National Hydrology Research Center, Saskatoon, Saskatchewan. Stable isotope measurements were expressed as parts-per-thousand (‰) deviations from a known standard. All values are reported in δ (delta) notation according to:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (3.1)$$

where R_{sample} is the isotope ratio of the sample and $R_{standard}$ is the isotope ratio of the standard. International isotopic standards used for carbon and nitrogen were Vienna Pee Dee Belemnite (VPDB) and atmospheric Air, respectively. Analytical measurement error was estimated to be ± 0.1 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$ measurements.

3.2.6. Turnover rates and Discrimination Factors

Each tissue represents a different diet integration period in accordance with its elemental turnover rate (Tieszen et al. 1983, Hobson and Clark 1992a) which is in turn

related to the tissue's metabolic rate (Bearhop et al. 2002). Because metabolic rates vary between wild vs. captive birds and large vs. small birds (Nagy 1987, Hobson and Bairlein 2003), experimentally-derived elemental turnover rates were chosen from a species corresponding as closely as possible to the Yellow-rumped Warbler when species-specific values were not available. The diet integration period for each tissue used in this study, and the species from which these values were derived, are presented in Table 3.1.

For birds captured in spring, liver represented recent (migration) diet, whole blood and muscle represented late-winter to early migration diet, claws represented winter diet, and feathers represented late-winter diet. For birds captured in fall, liver represented late- summer or early-migration diet, whole blood and muscle represented late-summer diet, claws represented early-summer diet and feathers represented late-summer diet. Bone collagen represented a lifetime average diet in adults and was likely biased towards winter diet because migratory birds spend the majority of their annual cycle in these periods (Hunt and Flaspohler 1998, Norris et al. 2004). For young birds (i.e., hatch-year (HY) birds during fall migration), bone collagen isotope values would be biased toward diets during the period of growth.

A diet-tissue isotopic discrimination factor (hereafter referred to as 'discrimination factor') refers to the change in isotope value that occurs between diet and a particular tissue (Hobson and Clark 1992b). Experimentally-derived discrimination factors used in this study are listed in Table 3.2. Recent studies have demonstrated a strong correlation between isotopic values of feathers and claws (Clark et al. 2006, Hobson et al. 2006) however no studies have experimentally determined discrimination factors for claws. Nevertheless, $\delta^{13}C$ and $\delta^{15}N$ feather discrimination factors were applied to claws assuming both tissues have similar discrimination factors because they are both keratinous tissues. Pearson et al. (2003) calculated discrimination factors (Δ_{dt}) for whole blood from Yellow-rumped Warblers fed an insect-based diet (97% insect: $\delta^{15}N$ Δ_{dt} = 2.7 ‰, $\delta^{13}C$ Δ_{dt} = 2.2 ‰) and those fed a fruit-based diet (20% insect: $\delta^{15}N$ Δ_{dt} = 1.7 ‰, $\delta^{13}C$ Δ_{dt} = -1.2 ‰). Based on Pearson et al.'s (2003) study, it seemed appropriate to use their diet-specific discrimination factors in this study. Unfortunately, discrimination factors for the other tissues have only been determined

Table 3.1 Diet integration periods for blood, muscle, liver, feathers, claws and bone collagen. These are based on experimentally derived tissue turnover rates. The captive studies and the species from which turnover rates were determined are also presented.

Tissue	Diet Integration Period	Study Species	Reference
Blood	2 to 3 weeks	Garden Warbler	Hobson and Bairlein 2003
Muscle	2 to 3 weeks	Japanese Quail	Hobson and Clark 1992a
Liver	3 to 6 days	Japanese Quail	Hobson and Clark 1992a
Feathers	Period of growth	American Crow	Hobson and Clark 1992a
Claw	2 to 3 months	passerines	Bearhop et al. 2003
Bone Collagen	Lifetime average	Japanese Quail	Hobson and Clark 1992a

Table 3.2 $\delta^{13}C$ and $\delta^{15}N$ diet-tissue discrimination factors for blood, muscle, liver, feathers, claws and bone collagen derived from captive studies. Discrimination factors determined for birds fed a fruit diet and birds fed an insect diet are presented. Insect-diet discrimination factors were estimated for muscle, liver, and bone collagen.

	$\delta^{15}N$		$\delta^{13}C$			
Tissue	Fruits	Insects	Fruits	Insects	Species	Reference
Blood	1.7	2.7	-1.2	2.2	Yellow-rumped Warbler	Pearson et al. 2003
Muscle	2.8	3.8[a]	0.2	3.6[a]	Yellow-rumped Warbler	Podlesack and McWilliams 2006
Liver	3.5	4.5[a]	0.1	3.5[a]	Yellow-rumped Warbler	Podlesack and McWilliams 2006
Feathers	3.2	3.5	1.9	4.3	Yellow-rumped Warbler	Pearson et al. 2003
Claws	3.2[b]	3.5[b]	1.9[b]	4.3[b]	n/a*	n/a*
Bone Collagen	2.5	3.5[a]	2.7	6.1[a]	Japanese Quail	Hobson and Clark 1992a

*No studies have determined discrimination factors for claws.

[a] Values were calculated based on the relative difference between blood fruit-diet discrimination factors and blood insect-diet discrimination factors determined by Pearson et al. (2003).

[b] Claw discrimination factors are assumed to be the same as feathers because they are both keratinous tissues and no other information is available in the literature.

from birds fed a plant-based diet. As such, I assumed that the magnitude of the difference between the fruit-diet discrimination factor and the insect-diet discrimination factor of whole blood applied to differences for this dietary dichotomy for other tissues. Insect-diet discrimination factors were calculated for muscle, liver, bone collagen and feathers based on the relative difference of 1 ‰ and 3.4 ‰ for $\delta^{15}N$ and $\delta^{13}C$ values, respectively.

3.2.7. Statistical Analysis

Non-parametric statistical tests were used for some analyses where data were not normally distributed. I used Spearman's correlation analysis to examine correlations of stable isotope values between all two-way comparisons of blood, liver, muscle, bone collagen, claw, and feather tissues. A Kruskal-Wallace test was used to determine if there was a difference between tissue $\delta^{13}C$ and $\delta^{15}N$ values and Mann-Whitney U-tests to determine where the differences occurred. In order to make a direct comparison of stable isotope values between tissues, appropriate diet-tissue discrimination factors (see above) were applied to the $\delta^{13}C$ and $\delta^{15}N$ values of each tissue so that isotopic values were standardized to the diet isotope values. Furthermore, Mann-Whitney U-tests were also used to examine within-tissue seasonal comparisons of $\delta^{13}C$ and $\delta^{15}N$ values. Since bone collagen isotope values may reflect a bird's juvenile diet during bone growth (Mizutani et al. 1986), bone collagen $\delta^{13}C$ and $\delta^{15}N$ values were compared between age classes (i.e., HY/SY vs. AHY/ASY). No significant differences (Spring 2003 – $\delta^{15}N$: $t_{(12)} = -0.2$, $P = 0.9$, $\delta^{13}C$: $t_{(12)} = -0.9$, $P = 0.4$; Fall 2003 – $\delta^{15}N$: $t_{(37)} = -1.7$, $P = 0.09$, $\delta^{13}C$: $t_{(37)} = -0.7$, $P = 0.5$) were detected and so data from both age classes were pooled for all analyses involving collagen. When conducting multiple comparisons within a dataset, a Bonferroni correction (α/n) was used to adjust the alpha value (α) relative to the number of comparisons (n).

I estimated the relative contribution of fruits and insects to the diet of each individual bird using a two-endpoint mixing model (Herrera et al. 2005):

$$P_f = (D_t - D_i)/(D_f - D_i) \quad (3.2)$$

where P_f is the proportion of fruits in the diet, D_t is the isotopic value of the consumer tissue measured, and D_i and D_f are the consumer tissue isotopic values corresponding to

exclusive diets of insects and fruits, respectively. The proportion of each food source in an individual's diet was only estimated for birds caught during fall 2003 because insect and fruit samples represented food items available to Yellow-rumped Warblers during breeding season and fall migration (i.e., these components were not available for spring migrants). Boreal fruits and herbivorous boreal insects were used as the dietary endpoints. Dietary proportions were averaged for each tissue.

For each bird sampled during spring migration, tissue $\delta^{13}C$ and $\delta^{15}N$ values, corrected to diet using appropriate discrimination factors, were directly compared to isotopic values of southern bayberry. Since I only had one food source from the wintering grounds, I could not use a mixing model to evaluate the proportion of food sources represented in tissues collected in spring. Similar isotopic values between tissues corrected for isotopic discrimination and bayberry samples should indicate that a bird fed predominately on this food source or one with similar isotopic values.

I used Pearson's correlation to evaluate the relationship between tissue $\delta^{15}N$ and deuterium (δD) values. I was interested in determining whether Yellow-rumped Warblers wintering further north were more reliant on fruits. Only individuals birds that had both $\delta^{15}N$ and δD data were included in this analysis. δD values in precipitation vary with latitude along a southeast to northwest gradient across North America (Hobson and Wassenaar 1997). Feather δD values can be linked to latitude because they retain the isotopic signature of the local environment which in turn reflects the isotopic composition of rainfall at the latitude where feathers were grown (Chamberlain et al. 1997, Hobson and Wassenaar 1997, Wassenaar and Hobson 2001). As such, δD values of greater coverts (feathers grown in winter) reflected the wintering ground latitude of Yellow-rumped Warblers.

3.3. RESULTS

3.3.1. Correlation of isotope values among tissues

During spring migration, there were no significant correlations between $\delta^{15}N$ and $\delta^{13}C$ values of different tissues (Table 3.3).

Table 3.3 Spearman's correlation results of all two-way comparisons of $\delta^{15}N$ and $\delta^{13}C$ for tissues collected from Yellow-rumped Warblers in *spring* 2003 at the Delta Marsh Bird Observatory, MB.

			$\delta^{15}N$		$\delta^{13}C$	
Tissue 1	Tissue 2	n	r	P[a]	r	P[a]
Muscle	Liver	21	0.01	1.0	0.2	0.5
Muscle	Bone collagen	21	0.03	0.9	0.2	0.5
Muscle	Claws	21	0.1	0.7	0.6	0.005
Muscle	Blood	4	-0.4	0.6	0.4	0.6
Muscle	Greater coverts	6	0.5	0.3	0.3	0.6
Liver	Bone collagen	21	0.2	0.5	0.01	1.0
Liver	Claws	21	0.05	0.8	0.2	0.4
Liver	Blood	4	0.4	0.6	0.8	0.2
Liver	Greater coverts	6	-0.7	0.2	0.4	0.4
Bone collagen	Claws	21	0.3	0.2	0.5	0.02
Bone collagen	Blood	4	0.4	0.6	0.7	0.3
Bone collagen	Greater coverts	6	0.2	0.7	0.09	0.9
Claws	Blood	4	0.0	1.0	0.6	0.4
Claws	Greater coverts	6	-0.1	0.8	0.7	0.2
Blood	Greater coverts	2	n/a	n/a	n/a	n/a

n/a = sample size is too small

[a]Bonferroni correction, $\alpha = 0.004$

During fall migration, 10 of 15 and 6 of 15 two-way comparisons were positively correlated for $\delta^{15}N$ and $\delta^{13}C$ values, respectively (Table 3.4).

3.3.2. Tissue Comparisons

To directly compare different tissues, $\delta^{13}C$ and $\delta^{15}N$ values were corrected using fruit-diet discrimination factors and insect-diet discrimination factors. It was not known which of these discrimination factors was more applicable to birds used in this study (i.e., true diet was unknown), so both discrimination factors were applied to determine if different discrimination factors would affect the outcome of these analyses and the overall conclusions drawn from these data.

3.3.2.1. Spring

Corrected bird $\delta^{15}N$ and $\delta^{13}C$ tissue values to equivalent fruit diets differed among tissues (Kruskal-Wallis, $\delta^{15}N$: $X^2_{(5, 98)} = 32.5$, $P < 0.001$; $\delta^{13}C$: $X^2_{(5, 98)} = 23.7$, $P < 0.001$). This was also the case for tissues corrected to corresponding insect diets (Kruskal-Wallis, $\delta^{15}N$: $X^2_{(5, 98)} = 21.8$, $P = 0.001$; $\delta^{13}C$: $X^2_{(5, 98)} = 30.0$, $P < 0.001$). Few of the post-hoc, two-way comparisons between tissues for $\delta^{15}N$ and $\delta^{13}C$ values were significantly different for tissues corrected with fruit-based (Table 3.5, Fig. 3.1A and Fig. 3.2A) and insect-based discrimination factors (Table 3.6, Fig. 3.1B and Fig. 3.2B).

3.3.2.2. Fall

During fall, there were significant differences in $\delta^{15}N$ and $\delta^{13}C$ values for tissues corrected with fruit-diet (Kruskal-Wallis, $\delta^{15}N$: $X^2_{(5, 231)} = 58.8$, $P < 0.001$; $\delta^{13}C$: $X^2_{(5, 231)} = 133.6$, $P < 0.001$) and insect-diet discrimination factors (Kruskal-Wallis, $\delta^{15}N$: $X^2_{(5, 231)} = 34.4$, $P < 0.001$; $\delta^{13}C$: $X^2_{(5, 231)} = 124.6$, $P < 0.001$). The majority of two-way comparisons between tissues for $\delta^{13}C$ values were significantly different for tissues corrected with fruit-diet (Table 3.5, Fig. 3.1A and Fig. 3.2A) and insect-diet discrimination factors (Table 3.6, Fig. 3.1B and Fig. 3.2B), but few two-way comparisons between $\delta^{15}N$ values of tissues were significantly different.

Table 3.4 Spearman's correlation results of all two-way comparisons of $\delta^{15}N$ and $\delta^{13}C$ for tissues collected from Yellow-rumped Warblers in *fall* 2003 at the Delta Marsh Bird Observatory, Manitoba, Canada.

			$\delta^{15}N$		$\delta^{13}C$	
Tissue 1	Tissue 2	n	r	P[a]	r	P[a]
Muscle	Liver	39	0.8	**<0.001**	0.8	**<0.001**
Muscle	Bone collagen	39	0.6	**<0.001**	0.3	0.04
Muscle	Claws	39	0.8	**<0.001**	0.4	0.01
Muscle	Blood	36	0.9	0.02	0.8	**<0.001**
Muscle	Tail feather	39	0.5	0.003	0.1	0.5
Liver	Bone collagen	39	0.4	0.009	0.2	0.3
Liver	Claws	39	0.6	**<0.001**	0.1	0.5
Liver	Blood	36	0.9	**<0.001**	0.7	**<0.001**
Liver	Tail feather	39	0.2	0.1	-0.06	0.7
Bone collagen	Claws	39	0.8	**<0.001**	0.6	**<0.001**
Bone collagen	Blood	36	0.7	**<0.001**	0.5	0.005
Bone collagen	Tail feather	39	0.8	**<0.001**	0.5	**0.001**
Claws	Blood	36	0.8	**<0.001**	0.5	0.003
Claws	Tail feather	39	0.7	**<0.001**	0.5	**0.001**
Blood	Tail feather	36	0.4	0.01	0.07	0.7

[a]Bonferroni correction, $\alpha = 0.003$; significant results in bold

Table 3.5 Mann-Whitney post-hoc tissue comparisons. Stable nitrogen and carbon isotope values were corrected using *fruit-diet* discrimination factors prior to analysis. Data were collected from Yellow-rumped Warblers migrating through the Delta Marsh Bird Observatory, MB, during spring and fall migration of 2003.

			$\delta^{15}N$		$\delta^{13}C$	
Season	Tissue 1	Tissue 2	M-W U	P[a]	M-W U	P[a]
Spring	Muscle	Liver	169	0.2	185	0.4
	Muscle	Bone collagen	121	0.01	105	0.004
	Muscle	Claws	71	**<0.001**	191	0.5
	Muscle	Blood	72	0.9	18	0.003
	Muscle	Greater coverts	28	0.02	55	0.3
	Liver	Bone collagen	76	**<0.001**	80	**<0.001**
	Liver	Claws	58	**<0.001**	168	0.2
	Liver	Blood	54	0.3	23	0.007
	Liver	Greater coverts	20	0.005	47	0.2
	Bone collagen	Claws	120	0.01	174	0.2
	Bone collagen	Blood	41	0.09	5	**<0.001**
	Bone collagen	Greater coverts	54	0.3	49	0.2
	Claws	Blood	30	0.02	21	0.005
	Claws	Greater coverts	39	0.07	73	1.0
	Blood	Greater coverts	13	0.1	3	0.006
Fall	Muscle	Liver	482	0.005	523	0.02
	Muscle	Bone collagen	624	0.2	181	**<0.001**
	Muscle	Claws	272	**<0.001**	491	0.007
	Muscle	Blood	561	0.1	62	**<0.001**
	Muscle	Tail feather	677	0.4	256	**<0.001**
	Liver	Bone collagen	620	0.2	137	**<0.001**
	Liver	Claws	143	**<0.001**	367	**<0.001**
	Liver	Blood	576	0.2	166	**<0.001**
	Liver	Tail feather	413	**0.001**	195	**<0.001**
	Bone collagen	Claws	204	**<0.001**	325	**<0.001**
	Bone collagen	Blood	683	0.8	7	**<0.001**
	Bone collagen	Tail feather	528	0.02	693	0.5
	Claws	Blood	192	**<0.001**	48	**<0.001**
	Claws	Tail feather	375	**<0.001**	402	**<0.001**
	Blood	Tail feather	503	0.04	18	**<0.001**

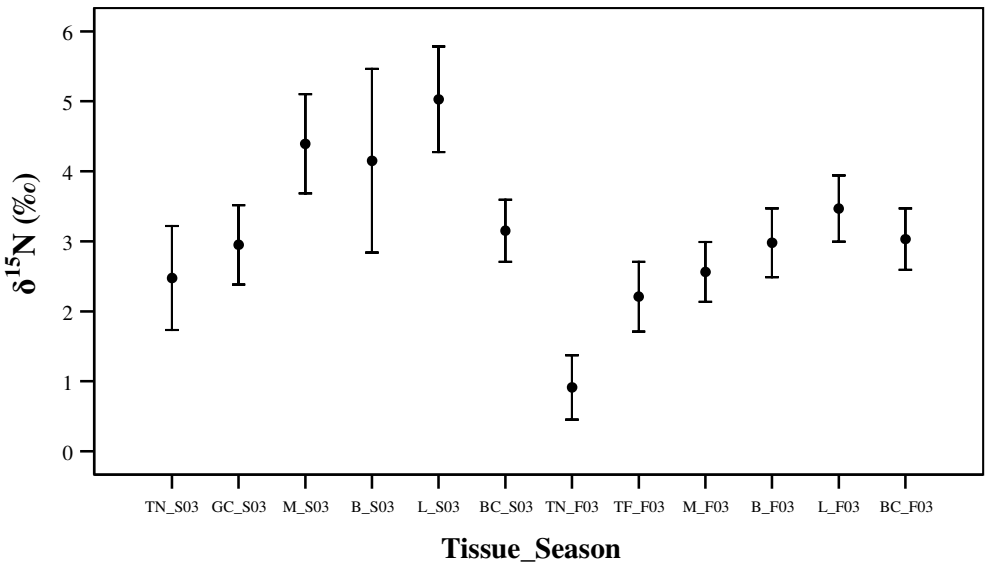
[a]Bonferroni correction, $\alpha = 0.003$; significant results in bold

Table 3.6 Mann-Whitney post-hoc tissue comparisons. Stable nitrogen and carbon isotope values were corrected using *insect-diet* discrimination factors prior to analysis. Tissues were collected from Yellow-rumped Warblers migrating through the Delta Marsh Bird Observatory during spring and fall migration of 2003.

			$\delta^{15}N$		$\delta^{13}C$	
Season	Tissue 1	Tissue 2	M-W U	P[a]	M-W U	P[a]
Spring	Muscle	Liver	169	0.2	185	0.4
	Muscle	Bone collagen	121	0.01	105	0.004
	Muscle	Claws	113	0.007	148	0.07
	Muscle	Blood	72	0.9	18	0.003
	Muscle	Greater coverts	55	0.3	42	0.09
	Liver	Bone collagen	76	**<0.001**	80	**<0.001**
	Liver	Claws	93	**0.001**	175	0.3
	Liver	Blood	54	0.3	23	0.007
	Liver	Greater coverts	33	0.03	55	0.3
	Bone collagen	Claws	176	0.3	81	**<0.001**
	Bone collagen	Blood	41	0.09	5	**<0.001**
	Bone collagen	Greater coverts	47	0.2	10	**0.001**
	Claws	Blood	44	0.1	50	0.2
	Claws	Greater coverts	39	0.07	73	1.0
	Blood	Greater coverts	16	0.3	11	0.09
Fall	Muscle	Liver	482	0.005	523	0.02
	Muscle	Bone collagen	624	0.2	181	**<0.001**
	Muscle	Claws	465	0.003	248	**<0.001**
	Muscle	Blood	561	0.1	62	**<0.001**
	Muscle	Tail feather	626	0.2	679	0.4
	Liver	Bone collagen	620	0.2	137	**<0.001**
	Liver	Claws	251	**<0.001**	525	0.02
	Liver	Blood	576	0.2	166	**<0.001**
	Liver	Tail feather	616	0.1	616	0.1
	Bone collagen	Claws	358	**<0.001**	36	**<0.001**
	Bone collagen	Blood	683	0.8	7	**<0.001**
	Bone collagen	Tail feather	749	0.9	230	**<0.001**
	Claws	Blood	317	**<0.001**	280	**<0.001**
	Claws	Tail feather	375	**<0.001**	402	**<0.001**
	Blood	Tail feather	701	1.0	158	**<0.001**

[a]Bonferroni correction, $\alpha = 0.003$; significant results in bold

A)



B)

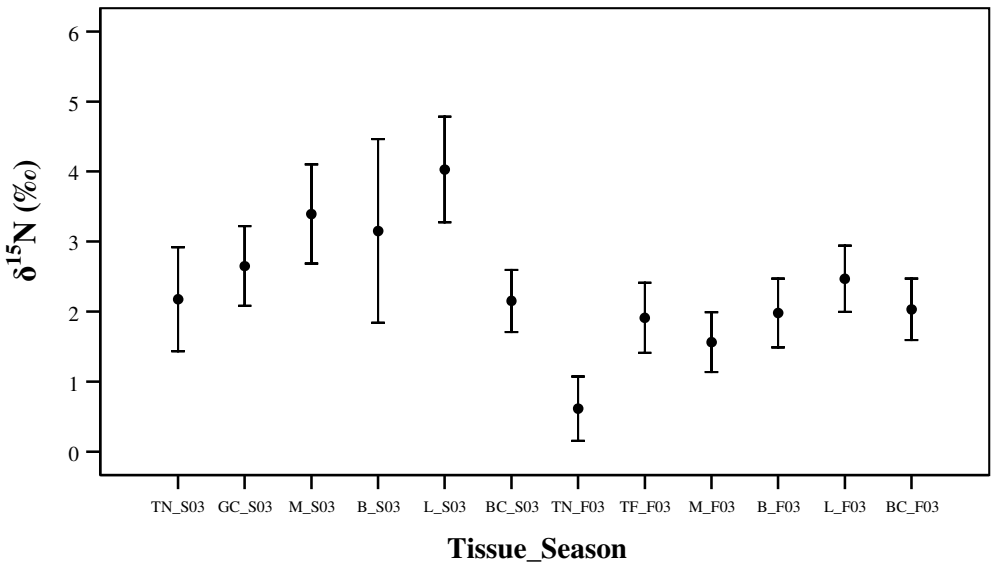


Figure 3.1 Mean $\delta^{15}N$ values (± 95% CI) of tissues collected from Yellow-rumped Warblers captured at the Delta Marsh Bird Observatory, Manitoba, Canada, during spring migration of 2003. Tissues were corrected to diet using (A) fruit-diet discrimination factors and (B) insect-diet discrimination factors to directly compare tissues to each other. L=liver, M=muscle, B=blood, F=feathers (GC=greater coverts in the spring, TF=tail feathers in the fall), TN=claws, BC=bone collagen, S03=spring migration 2003, F03=fall migration 2003.

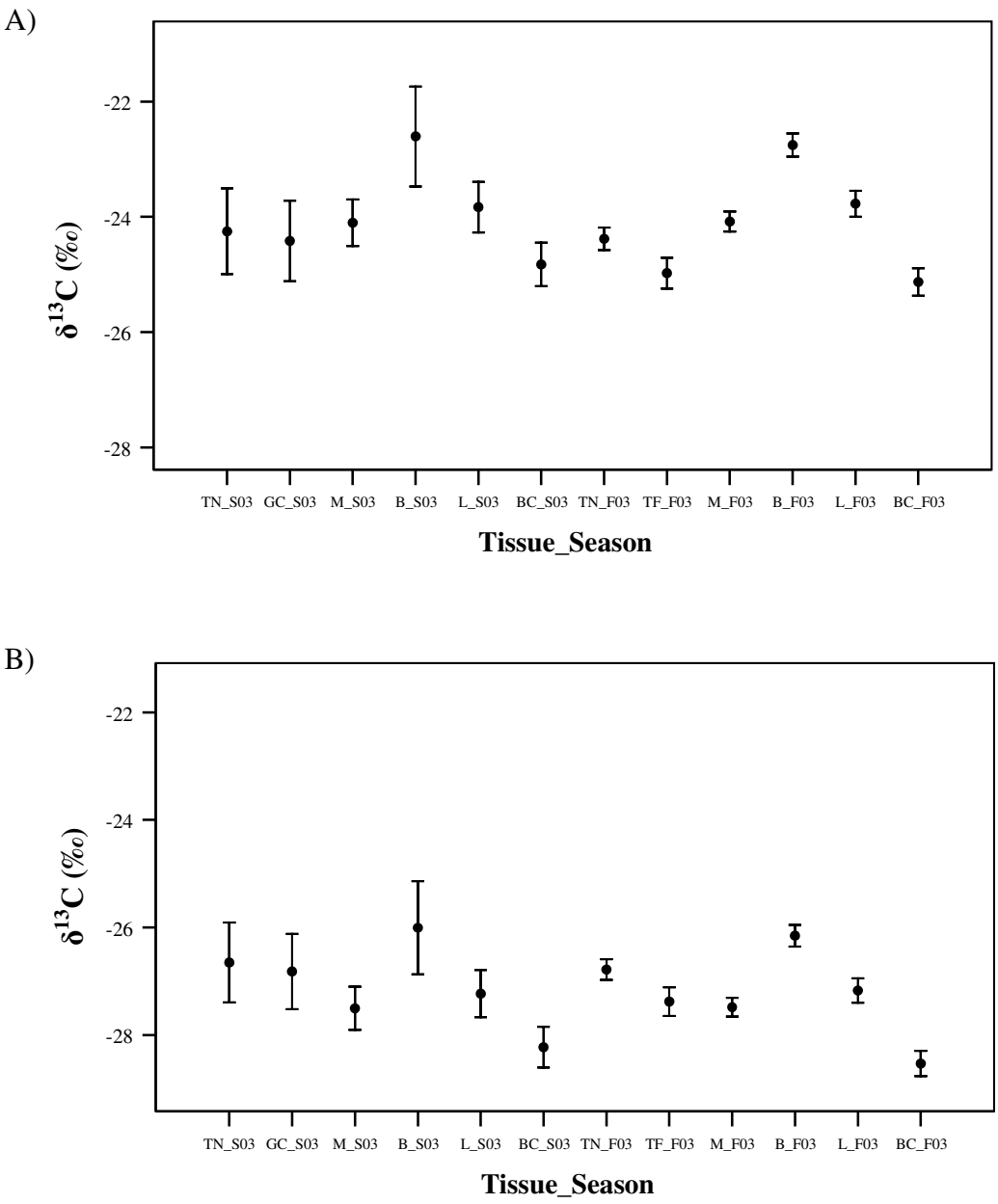


Figure 3.2 Mean $\delta^{13}C$ values (± 95% CI) of tissues collected from Yellow-rumped Warblers captured at the Delta Marsh Bird Observatory, Manitoba, Canada, during spring migration of 2003. Tissues were corrected to diet using (A) fruit-diet discrimination factors and (B) insect-diet discrimination factors to directly compare tissues to each other. L=liver, M=muscle, B=blood, F=feathers (GC=greater coverts in the spring, TF=tail feathers in the fall), TN=claws, BC=bone collagen, S03=spring migration 2003, F03=fall migration 2003.

3.3.3. Seasonal Comparisons

Spring and fall $\delta^{15}N$ values were significantly different for liver (Mann-Whitney U, $\delta^{15}N$: $U_{0.05(2),\,21,\,39} = 198.0$, $P = 0.001$), muscle ($\delta^{15}N$: $U_{0.05(2),\,21,\,39} = 149.5$, $P < 0.001$), and claws ($\delta^{15}N$: $U_{0.05(2),\,21,\,39} = 178.0$, $P < 0.001$). None of the seasonal tissue comparisons for carbon were significant. In general, spring $\delta^{15}N$ values were consistently higher than fall $\delta^{15}N$ values for all tissues (Fig. 3.3).

3.3.4. Relationship Between $\delta^{15}N$, $\delta^{13}C$ and δD

The relationship between $\delta^{15}N$ and δD and between $\delta^{13}C$ and δD was evaluated using Pearson's correlation for individuals that had data for both isotopes. There was no significant relationship between $\delta^{15}N$, $\delta^{13}C$ and δD values for each tissue with the exception of blood ($\delta^{13}C$: $r^2 = 0.8$, $P = 0.001$).

3.3.5. Contribution of Fruits and Insects

Boreal fruits and herbivorous insects available to Yellow-rumped Warblers differed in their average $\delta^{15}N$ values (t-test, t=2.7, df=97, P=0.009), with insects having higher $\delta^{15}N$ values (mean = 3.0 ‰) than fruits (mean = 1.1 ‰), but not in their average $\delta^{13}C$ (t-test t=0.1, df=33, P=0.9). $\delta^{15}N$ values ranged from -2.1 to 7.4 ‰ in fruits and -2.8 to 9.4 ‰ in insects, while $\delta^{13}C$ values ranged from -24.2 to -29.4 ‰ in fruits and -22.5 to -32.4 ‰ in insects. $\delta^{13}C$ values were characteristic of C_3 food webs in North America's temperate zone. Because $\delta^{13}C$ values of each food type were not isotopically distinct, only $\delta^{15}N$ values were used to estimate the contribution of fruits and insects to the diet of Yellow-rumped Warblers. This analysis was conducted for birds captured in fall only. The estimated average contribution of fruits to the diet of Yellow-rumped Warblers was similar across all tissues (Fig. 3.4). This trend was also observed in the estimated average contribution of insects although claws had a demonstrably lower predicted insect contribution than other tissues. The mixing model was not able to predict proportions of fruits and insects for 19, 11, 22, 18 and 13 individuals out of 40 for liver, muscle, claw, tail feather, and bone collagen, respectively, and 12 individuals out of 37 for blood. For the remaining birds, the estimated average contribution of insects to the diet was higher than the contribution of fruits for calculations based on

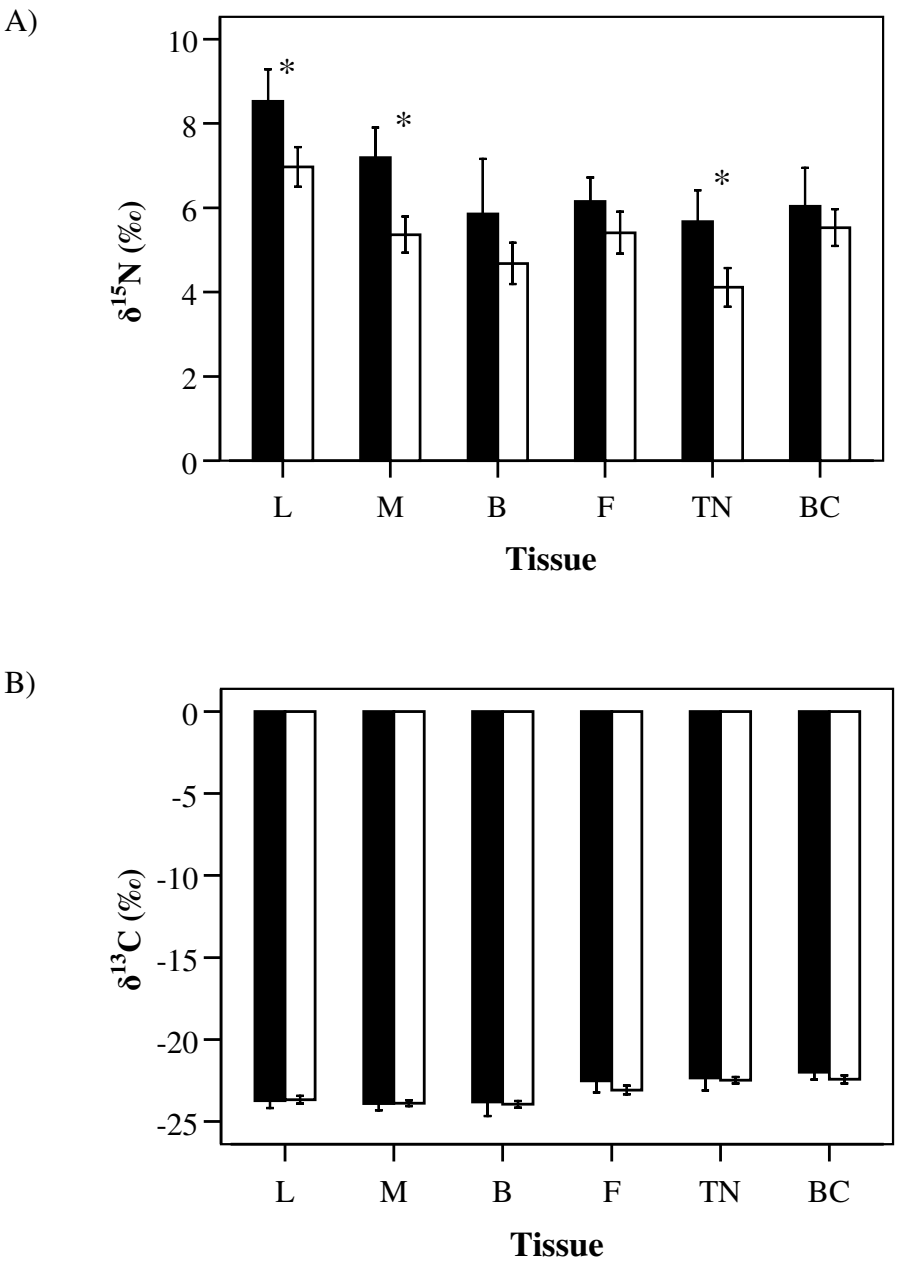


Figure 3.3 Within-tissue seasonal comparisons of mean $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values (± 95% CI) of tissues collected from Yellow-rumped Warblers captured at the Delta Marsh Bird Observatory, Manitoba, Canada, during spring (black bars) and fall (open bars) migration of 2003. L=liver, M=muscle, B=blood, F=feathers (GC=greater coverts in the spring, TF=tail feathers in the fall), TN=claws, BC=bone collagen.

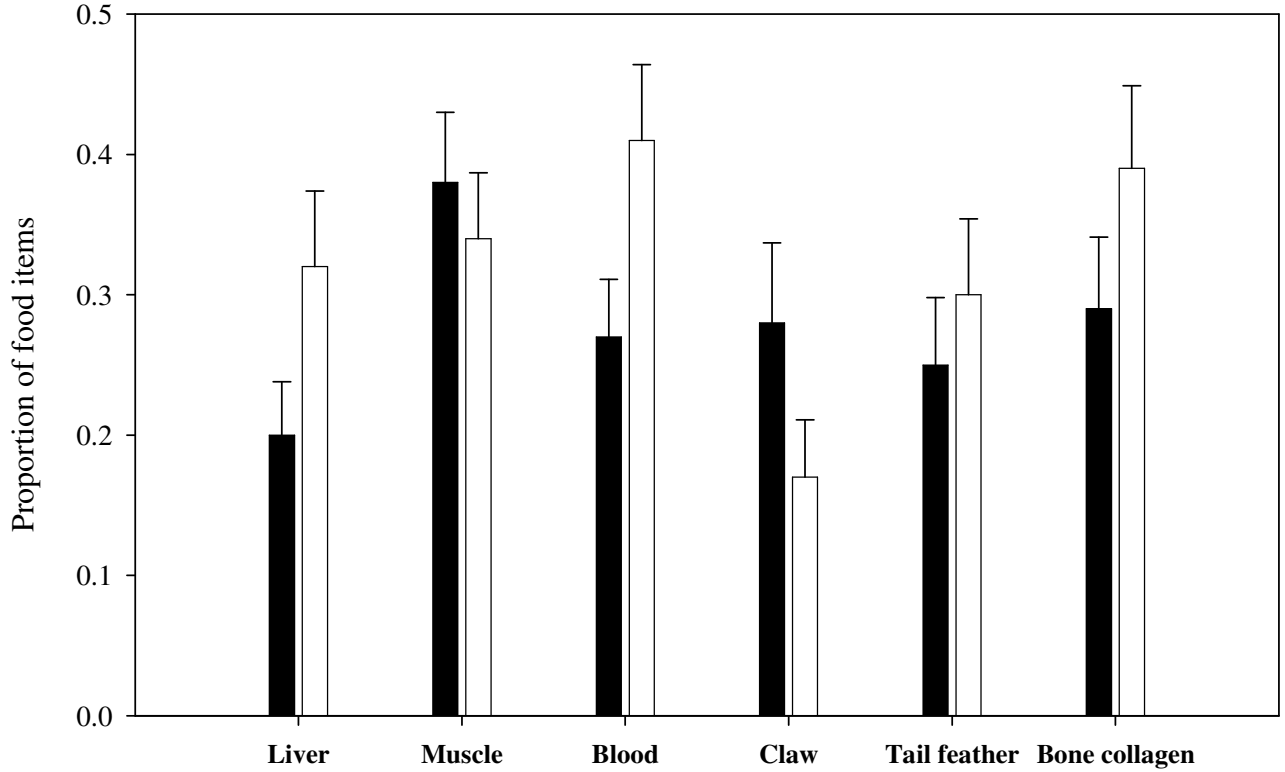


Figure 3.4 Proportion (± SE) of fruits (black bars) and insects (open bars) to the diet of Yellow-rumped Warblers migrating through the Delta Marsh Bird Observatory, Manitoba, Canada. Values were derived from a two-endpoint mixing model using only $\delta^{15}N$ values. Fruits and herbivorous insects collected in the boreal forest were used as the two-endpoints (fruit $\delta^{15}N$ = 1.1 ‰; insect $\delta^{15}N$ = 3.0 ‰). See Table 3.2 for discrimination factors applied to each tissue.

liver (fruits vs. insects = 20% vs. 32%), blood (27% vs. 41%), tail feather (25% vs. 30%), and bone collagen (29% vs. 39%). Muscle (38% vs. 34%) and claw (28% vs. 17%) tissues indicated a higher proportion of fruits in the diet of Yellow-rumped Warblers during their respective diet integration periods. Liver, muscle and blood were assumed to represent diet during fall migration but did not have a higher proportion of fruits as expected, with the exception of muscle. Claws were assumed to represent an insectivorous summer diet but indicated a higher proportion of fruits at that time of year. Based on the bone collagen data, there appeared to be almost equivalent amounts of insects and fruits in the overall diet of Yellow-rumped Warblers. Bayberries had significantly lower $\delta^{15}N$ and $\delta^{13}C$ values than the diet equivalent isotope values in all tissues (Fig. 3.5).

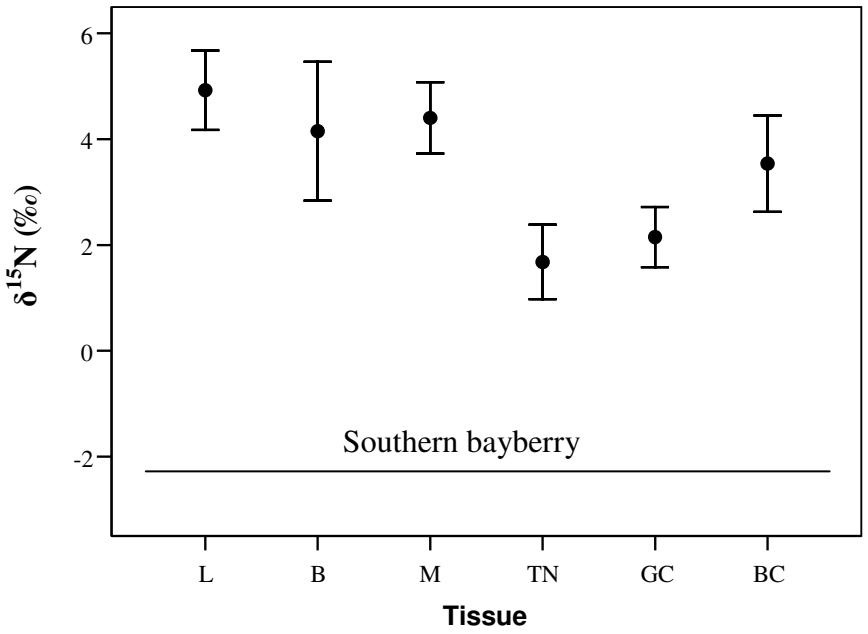
3.4. DISCUSSION

Although frugivory in Yellow-rumped Warblers is a well known aspect of their life history, it has proven difficult to demonstrate isotopically their reliance on fruits during fall and spring migration through southern Manitoba in 2003.

3.4.1. Correlations of isotope dietary information among tissues

Elemental turnover rate depends primarily on metabolism and will vary between tissues. My assumption was that if two tissues represent a similar diet integration period (i.e., similar turnover rates) then their stable isotope values and derived dietary information should be correlated. Overall, results did not follow my predictions. Based on available information in the literature, I predicted that muscle and whole blood $\delta^{15}N$ and $\delta^{13}C$ values would be correlated because similar turnover rates of approximately 2-3 weeks have been reported (Hobson and Clark 1992a, Hobson and Bairlein 2003). Muscle and blood stable isotope values were only significantly correlated for $\delta^{13}C$ values during fall migration. Overall, inferred diet was uniform across time periods in fall suggesting there was no major isotopic change in diet and that tissues representing short time periods (e.g. liver) reflected the same diet than tissues representing longer time periods (e.g. bone collagen). During spring, diet integration periods of different tissues did not correlate well indicating that short-term diet differed from long-term diet.

A)



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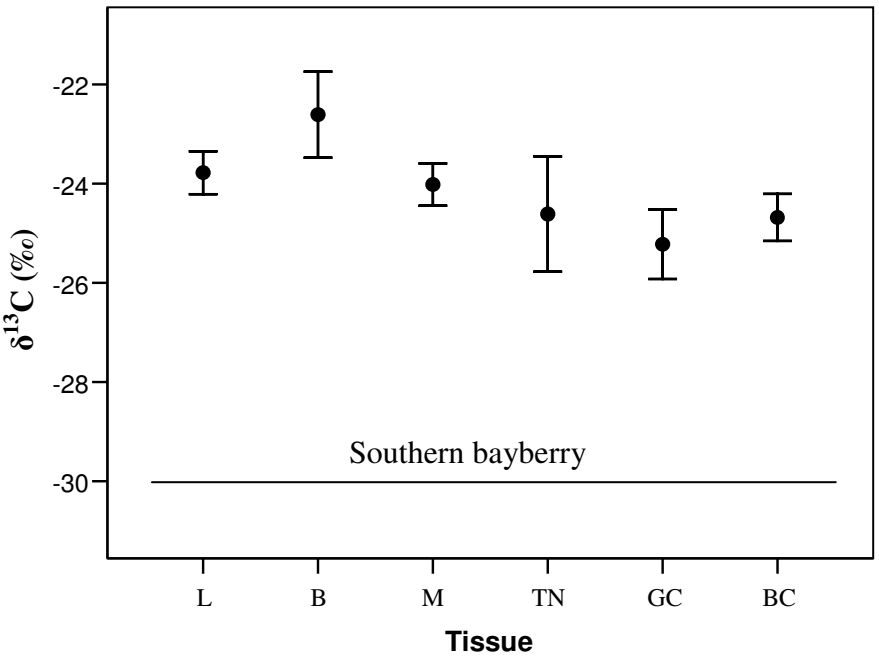

Figure 3.5 Mean $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values (± 95% CI) of tissues collected from Yellow-rumped Warblers captured at the Delta Marsh Bird Observatory, MB, during spring migration of 2003 and of southern bayberry (*Myrica cerifera*) collected in Florida during winter in 2006 (depicted as a solid line). L=liver, M=muscle, B=blood, GC= greater coverts, TN=claws, BC=bone collagen.

Using a wind tunnel to exercise birds to simulate the degree of activity of wild migratory songbirds, Hobson and Yohannes (2007) found that the elemental turnover rate of blood did not differ between non-exercised and exercised birds and that turnover rates from captive, unexercised birds provide a reasonable diet integration estimate. Nevertheless, this may not apply to other tissues considering that metabolic rates of captive animals have been shown to differ from those of wild animals (Nagy 1987) therefore caution is warranted when applying experimentally-derived turnover rates.

3.4.2. Annual Diet Variations

The assumption was made that tissue $\delta^{15}N$ values would reflect the trophic level at which birds were feeding with low $\delta^{15}N$ values suggesting a fruit-dominated diet and high $\delta^{15}N$ values suggesting an insect-dominated diet. When conducting within-tissue seasonal comparisons, the dietary trend of fruits dominating fall migration and winter diet and insects dominating summer and spring migration diet was well reflected in liver, muscle, and blood, tissues representing migration periods, which had lower $\delta^{15}N$ values in fall compared to spring. On the other hand, claws and feathers collected during spring and fall migration, tissues assumed to reflect winter and summer periods, respectively, had unexpectedly higher $\delta^{15}N$ values during winter compared to summer. As expected, there was no difference in $\delta^{13}C$ values for all tissues collected during spring and fall migration suggesting that these birds fed in C_3 environments throughout the year. Bone collagen $\delta^{15}N$ values did not significantly differ between birds sampled during both migration periods suggesting similar average lifetime diets. This could imply that the Yellow-rumped Warblers migrating through the DMBO either belonged to the same population and therefore had access to the same food sources or that the diet of different populations of migratory Yellow-rumped Warblers was similar over time and so the choice of food sources did not vary dramatically between populations.

The winter diet of Yellow-rumped Warblers has been well documented because of their unique dependence/attraction to bayberry fruits and their ability to digest the fruits' waxy coating (Place and Stiles 1992). On the other hand, the Yellow-rumped Warbler's diet during the breeding season is not well studied and most studies that report a summer diet of almost exclusively insects are old (see Hunt and Flaspohler 1998). Up-

to-date studies documenting the diet of Yellow-rumped Warblers during the breeding season are needed and that information would be helpful for better interpretation of isotope signatures.

The diet of Yellow-rumped Warblers and the degree to which they utilize fruits vs. insects as food sources may also depend on where the sampled birds originated. Yellow-rumped Warblers wintering inland have been reported to eat less fruits than coastal birds (Yarborough and Johnston 1965). According to stable-hydrogen isotope data of feathers, Yellow-rumped Warblers wintered in the southeastern U.S. with the majority of birds located along coastal regions of Florida and Texas (see Appendix 1, Fig. A.1.2). According to Amundson et al. (2003b), a substantial part of the southeastern U.S. is dominated by agricultural land and coastal areas are becoming more populated. As such, habitats in these areas are likely to be enriched in ^{15}N because anthropogenic activities tend to add nitrogen to the environment by way of fertilizers and tilling soils (Riga et al. 1971, Vitousek et al. 1997, Amundson et al. 2003a). Nevertheless, $\delta^{15}N$ values of southern bayberries from Florida (Pearson, S.F. and Levey, D.J., pers. comm., mean (SD) = -2.3 (0.3) ‰ for $\delta^{15}N$, -30.0 (1.2) ‰ for $\delta^{13}C$) were comparable to the $\delta^{15}N$ values of some my boreal fruits. Therefore, if Yellow-rumped Warblers are indeed feeding on bayberries in the southeastern United States during winter, ^{15}N enrichment due to anthropogenic activities may not be contributing to high $\delta^{15}N$ values in tissues representing winter diet. Yellow-rumped Warblers migrating through the DMBO in the fall of 2003 bred primarily in the mid-boreal to high-boreal forest in North America (see Appendix 1, Fig. A.1.2). These results are in accordance with results from Dunn et al. (2006).

The post-hoc tissue comparison results illustrate that whether a significant difference will be detected when comparing two tissues can differ based on which discrimination factor is used. How diet-tissue discrimination factors differ between a fruit diet and an insect diet remains a grey area. If discrimination factors are higher in birds feeding on fruits (i.e. a diet with low %N, low protein quality, and high C:N ratio) compared to discrimination factors between birds and insects (Robbins et al. 2005), then the proportion of fruits in a bird's diet could be underestimated as these birds would have high tissue $\delta^{15}N$ values inconsistent with a fruit diet. Pearson et al. (2003) was the

only study to examine discrimination factors in birds fed diets with varying proportions of fruits and insects. It is not known whether the relative difference between the blood fruit and insect discrimination factors in Pearson et al. (2003)'s paper correctly applies to other tissues. Also, isotopic discrimination factors have not been determined for claws yet. Thus, further studies like Pearson et al.'s (2003) are needed to determine the discrimination factors of multiple tissues from songbirds fed various proportions of fruits and insects.

3.4.3. Relationship between $\delta^{15}N$, $\delta^{13}C$ and δD

Few individuals had both $\delta^{15}N$, $\delta^{13}C$ and δD data available for each tissue therefore sample sizes were fairly small for this analysis, limiting my powers of interpretation. In general, the latitude at which birds wintered did not seem to correlate with diet. Yellow-rumped Warblers are one of the only wood warblers capable of wintering so far north in the U.S. It has been suggested that they can achieve this partly because of their larger body size but especially because of their association with bayberries and their ability to digest the wax coating of these fruits, providing them with a food source that other wood warblers are not able to exploit (Wilz and Giampa 1978, Morse 1989, Hunt and Flaspohler 1998). My data do not support the assumption that Yellow-rumped Warblers wintering further north ate more fruits than those wintering further south since there was no relationship between winter feather δD and $\delta^{15}N$ values.

3.4.4. Contribution of fruits and insects

Reconstructing the diet of a migratory passerine has proven to be more complex than previously thought. Fruit and insect end-points of the mixing model were not isotopically distinct from one another for carbon and so stable-carbon isotope data were not used in the diet reconstruction. This is not very surprising since limited variation exists in temperate C_3 environments in comparison to the tropics where within the same food web, C_3 and C_4 plants can be found (Tieszen and Boutton 1989, Herrera et al. 2001b, Herrera et al. 2003).

Unexpectedly, diet reflected by blood collected in fall included more insects than fruits, contrary to muscle tissue values. Since blood represents migration diet, I

expected more fruits in the diet of Yellow-rumped Warblers during fall migration. Also, because muscle and blood have similar diet integration periods, I anticipated results to be similar for both tissues. The elemental turnover rate of blood appears to be similar between captive and free-living birds (Hobson and Yohannes 2007) however it is not known whether that also applies to other tissues. Therefore, the higher metabolism of wild birds may affect the elemental turnover rates of other tissues causing them to represent diet over a shorter time period. This may explain why muscle tissue reflected a diet made up of more fruits than insects relative to blood.

For bone collagen, although there was a small difference in the percentage of fruits and insects predicted in their diet, both food sources played an important role in the diet of Yellow-rumped Warblers during their lifetime. I predicted that fruits would be more prominently reflected in liver during fall migration because this tissue should represent diet days prior to capture. Unexpectedly, the contribution of insects was much higher than that of fruits in liver from fall-captured birds. As Yellow-rumped Warblers approached the DMBO, perhaps they had begun feeding on berries from the surrounding agricultural areas. This would account for the higher $\delta^{15}N$ values as food items in these landscapes are typically more enriched in $\delta^{15}N$, with fruit $\delta^{15}N$ values potentially resembling insect $\delta^{15}N$ signatures (see Chapter 1), making it difficult to tease apart these two food sources. As such, this model may be under-estimating the use of fruits during fall migration. Alternatively, birds may not have switched their diet from insects to fruits when they were caught during fall migration.

Claws and feathers were expected to reflect an insect diet throughout the breeding season. Tail feathers reflected somewhat more insects than fruits in the diet of Yellow-rumped Warblers post-breeding. However, claws indicated there were more fruits relative to insects in the Yellow-rumped Warbler's diet during the summer. No studies have reported that fruits are part of the breeding season diet of Yellow-rumped Warblers (Hunt and Flaspohler 1998). Thus, the diet-tissue discrimination factors used in the mixing model, especially for claws, were likely not appropriate.

The comparison of $\delta^{15}N$ and $\delta^{13}C$ values between bayberries and tissues indicated that Yellow-rumped Warblers were feeding on food sources that were comparatively enriched in ^{15}N and ^{13}C during winter and spring migration. Surprisingly,

it does not appear that the Yellow-rumped Warblers migrating through Delta Marsh fed substantially on bayberries during winter, a phenomenon that has been observed on many occasions (e.g., Hausman 1927, Wilz and Giampa 1978, Kwit et al. 2004a). However, there are few reports of this species feeding substantially on fruits during spring migration (Parrish 2000) so these results seem to indicate that birds may have switched to an insect diet prior to migration (Yarborough and Johnston 1965).

3.5. FUTURE RESEARCH NEEDS

The use of mixing models to reconstruct the diet of Yellow-rumped Warblers was based on the assumptions that: 1) diet integration periods based on captive-derived turnover rates are accurate, 2) current life history information regarding its diet is accurate and 3) correct discrimination factors were used and/or estimates of insect-diet discrimination factors were correctly calculated. Studies to qualify and quantify the diet of Yellow-rumped Warblers during its breeding season would facilitate the interpretation of stable isotope signatures observed in tissues reflecting diet in that part of their lifecycle. It is clear that we do not yet have a good enough understanding of diet-tissue discrimination factors for songbirds feeding on omnivorous diets. Pearson et al.'s (2003) study is the only one so far to provide a range of discrimination factors for birds fed diets with a varying percentage of fruits and insects. These values need to be validated with more studies. My assumption that the difference between fruit and insect diet discrimination factors for other tissues would follow the relative difference pattern observed in blood, based on Pearson et al. (2003), may be wrong. The work of Hobson and Bairlein (2003) and Robbins et al (2005) tend to undermine the Pearson study. Pearson et al. (2003) showed that discrimination factors decreased with decreasing dietary nitrogen concentration (i.e. high C:N diet). However, neither Hobson and Bairlein (2003) or Robbins et al. (2005) found a relationship between discrimination factors and a diet's C:N ratio. Future research should focus on more captive studies to gain a better understanding of turnover rates in active birds (see Hobson and Yohannes 2007) and diet-tissue discrimination factors in passerines, particularly between keratin and diet, and how these discrimination factors change between insect-based and fruit-based diets. Better information on the link between claws/feathers and diet is of

particular importance because these tissues are easily obtained, non-destructive tissues which are often selected by ecologists. Furthermore, more knowledge of how isotope values in insects and fruits change spatially is needed.

CHAPTER 4: SUMMARY AND SYNTHESIS

Seasonal diet shifts have been documented in several species of North American migratory songbirds (Wheelwright 1988, White and Stiles 1990, Parrish 1997, Parrish 2000). Generally, this dietary plasticity is observed as a shift from an insect diet during the breeding season to one incorporating fruits and other plant materials during migration and non-breeding periods. Little is known about the extent to which diet shifts occur in migratory songbirds due, in part, to the logistics of tracking songbirds throughout their annual cycle. Stable isotope analysis has proven to be successful when examining trophic relationships (Herrera et al. 2003) and diet (Haramis et al. 2001) but few studies have used this technique to study frugivory in omnivorous North American songbirds (Podlesak et al. 2005).

For my thesis research, I used stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses of tissues representing different time periods at various geographic locations from sixteen species of migratory songbirds, with a focus on Yellow-rumped Warblers, to evaluate the timing and extent of frugivory throughout their annual life cycle. Multiple tissues were sampled to examine dietary patterns of migratory songbirds as these represent different diet integration periods due to varying elemental turnover rates. Based on the assumption that tissue $\delta^{15}N$ values reflect the trophic level at which birds are feeding (low $\delta^{15}N$ = fruit diet, high $\delta^{15}N$ = insect diet), I expected that tissues representing fall migration (liver, blood, and muscle from fall-captured birds) and winter (greater coverts and claws from spring-captured birds) would have lower $\delta^{15}N$ values than tissues representing spring migration (liver, blood, and muscle from spring-captured birds) and summer (tail feathers and claws from fall-captured birds). $\delta^{13}C$ values show a slight increase with trophic level (Peterson and Fry 1987) but they are also indicative of feeding environments (e.g., C_3 vs. C_4, Hobson 2003) and possibly feeding latitude (Körner et al. 1991). When evaluating questions regarding all sixteen species of migratory songbirds, I used blood and claw $\delta^{13}C$ and $\delta^{15}N$ values only.

Although stable isotope analysis has been successful in various dietary studies (Hobson 1993, Hobson et al. 2000b, Haramis et al. 2001, Herrera et al. 2005, Podlesak et al. 2005), this is the first study to use this technique to examine frugivory in temperate North American migratory songbirds. As such, the nature of this research was mainly exploratory. In Chapter 2, I evaluated the usefulness of the stable isotope technique to distinguish between species classified as insectivores or omnivores as well as examine seasonal patterns in frugivory as reflected by $\delta^{15}N$ values. Species classified as insectivores and those classified as omnivores did not segregate isotopically as expected. Predicted seasonal patterns in $\delta^{15}N$ values were supported for blood values of Tree Swallows and the claw values of American Robins, Baltimore Orioles, Gray Catbirds, Least Flycatchers, and Warbling Vireos. However, for the remaining species, tissue $\delta^{15}N$ values did not change between seasons or a seasonal shift in $\delta^{15}N$ values in the opposite direction to my predictions was observed. Of note, American Robins, Cedar Waxwings, Yellow-rumped Warblers and Hermit Thrushes did not show a significant seasonal diet shift between spring and fall migration periods. Among insectivores, House Wrens and Magnolia Warblers unexpectedly showed seasonal differences in blood and claw $\delta^{15}N$ values. There was limited variation in tissue $\delta^{13}C$ values which could reflect either the small trophic effect of $\delta^{13}C$ in food webs and/or the isotopically homogenous $\delta^{13}C$ landscapes experienced by these birds.

Revision of guild classifications of most migratory songbirds is warranted and I believe it would be more appropriate to assign guilds by season to better reflect annual diet variations. As such, more study is needed to improve our knowledge of winter and migration diets of migratory songbirds. The inaccurate assessment of frugivory in these species or the invalid use of stable isotope analyses (i.e. using incorrect assumptions regarding isotopic differences between fruits and insects) are possible explanations for the lack of fit between my isotope values and species dietary expectations. As well, the overlap in isotopic values of agricultural fruits with boreal fruits and insects indicate that food web baselines are not isotopically segregated as expected and do not conform to a simple linear trophic-enrichment model. Anthropogenic activities, such as the input of fertilizer and cultivating fields for agriculture, can alter landscapes by artificially increasing the nitrogen content in food webs thereby complicating the interpretation of

$\delta^{15}N$ values. As reflected by my results, examining frugivory in North American migratory songbirds is a complex task and influenced by many factors. Complicating factors include the variety of food webs and landscapes migrant songbirds encounter throughout their annual life cycle, the considerable amount of variability among and between fruits and insects, and the uncertainty that remains about the true discrimination factors for these food sources. For these reasons, I caution against the use of stable isotope analysis to track frugivory in temperate North American migratory songbirds.

Diet shifts have been well documented in Yellow-rumped Warblers with fall migration and winter diets being dominated by fruits (Hausman 1927, Yarbrough and Johnston 1965, Wilz and Giampa 1978, Malmborg and Willson 1988, Parrish 1997, Suthers et al. 2000, Borgmann et al. 2004, Podlesak et al. 2005) and breeding and spring migration diets being dominated by insects (Bent 1953, Yarbrough and Johnston 1965, Parrish 2000). As such, I wanted to focus more closely on this species and examine annual diet variations using a multiple tissue, stable isotope approach. In Chapter 3, I explored correlations of isotope dietary information among tissues, evaluated annual diet variations and attempted to reconstruct the fall and summer diet of Yellow-rumped Warblers using a two-endpoint mixing model. With the exception of muscle and blood, tissues were not expected to be correlated because they had different turnover rates (i.e. different diet integration period). However, the majority of tissues collected from fall birds were significantly correlated, including muscle and blood. This suggests that there was no significant change in diet across time periods represented by fall-collected tissues. During spring, there was a poor correlation among diet integration periods of different tissues suggesting a major change in diet.

Within-tissue seasonal comparisons indicated that the expected dietary trend (fruits dominating fall migration and winter diets and insects dominating summer and spring migration diets) was reflected in liver, muscle and blood (all representing migration periods) which had lower $\delta^{15}N$ values in fall birds compared to spring birds. Claws and feathers from spring birds (reflect winter diet) and fall birds (reflect summer diet) had unexpectedly higher $\delta^{15}N$ values during winter compared to summer. As expected, there was no difference in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values between birds sampled during spring and fall migration periods suggesting similar average lifetime

diets. When comparing different tissues to each other across seasons, liver collected from fall birds had unexpectedly high $\delta^{15}N$ values whereas claws and feathers had unexpectedly low $\delta^{15}N$ values. $\delta^{13}C$ and $\delta^{15}N$ values were corrected using fruit and insect diet-tissue discrimination factors in order to make a direct comparison between tissues. Little is known about which discrimination factors are more applicable to omnivorous birds therefore both were applied to evaluate if different discrimination factors would affect the overall outcome of the results.

Mixing model results for $\delta^{15}N$ values indicated a higher proportion of insects in the diet of Yellow-rumped Warblers caught during fall migration for all tissues except muscle and claws. I predicted that muscle and blood would reflect similar proportions of fruits and insects because they have similar diet integration periods and both represent fall migration diet. However, blood reflected a diet that included more insects than fruits, contrary to the results for muscle. Liver should represent diet days prior to capture therefore I expected liver to reflect a higher proportion of fruits. Unexpectedly, the contribution of fruits was significantly lower than that of insects. Claws and feathers, reflecting breeding season diet, were expected to reflect an insect diet. Indeed, tail feathers indicated slightly more insects than fruits in the diet of Yellow-rumped Warblers during moult. Conversely, claws reflected a summer diet composed of significantly more fruits than insects. Bone collagen $\delta^{15}N$ values indicated that both fruits and insects play an important role in the lifetime diet of Yellow-rumped Warblers with insects occupying a higher proportion than fruits. No studies have indicated that the breeding season diet of Yellow-rumped Warblers includes fruits (Hunt and Flaspohler 1998).

Reconstructing the diet of omnivorous migratory songbirds, like the Yellow-rumped Warbler, has proven to be fairly complicated and potentially inaccurate with the information that is currently available. A number of factors should be taken into consideration when interpreting my results. I assumed that experimentally-derived elemental turnover rates, and their corresponding diet integration periods, were accurate. More captive studies should be the focus of future research to better understand turnover rates in various tissues of active birds (see Hobson and Yohannes 2007). Furthermore, I assumed that the available dietary information was accurate. Although many studies

have evaluated the Yellow-rumped Warbler's winter and fall migration diet, there should be more recent studies conducted to qualify and quantify their breeding season and spring migration diet, two periods of their life cycle which have not received as much attention in terms of diet. The interpretation of stable isotope values observed in tissues which reflect summer and spring diet would benefit from additional dietary information. Another assumption was that the correct diet-tissue discrimination factors were applied tissue $\delta^{13}C$ and $\delta^{15}N$ values and that the chosen method of calculating insect diet discrimination factors was valid. Pearson et al. (2003) fed Yellow-rumped Warblers different diets varying in their fruit and insect content and calculated a range of discrimination factors. To my knowledge, this is the only study to have determined different discrimination factors depending on whether birds were fed predominately fruits or insects. More studies of this kind are needed to validate these values and to determine proper discrimination factors for other tissues as well. Future research should also focus on better understanding diet-tissue discrimination factors in omnivorous passerines, particularly between keratin and diet, and how discrimination factors change between insect-based and fruit-based diets. For example, according to my data, a muscle discrimination factor 30% lower than the current discrimination factor reduced the estimated proportion of fruits and insects to 18% and 30%, respectively, while a 30% increase in the muscle discrimination factor changed the estimated proportion of fruits and insects to 41% and 21%, respectively. Claws and feathers are non-destructive tissues which are often selected by ecologists therefore a greater understanding of the link between keratin-based tissues and diet is particularly important. My thesis research is a first step in exploring how avian omnivorous diets can be studied using stable isotope analysis, a technique increasingly applied by scientists to answer ecological questions. Furthermore, my findings open the door for future studies to address specific issues for which more information is needed if biologists are to be more successful at applying this technique to studying frugivory in migrant songbirds within landscapes and possibly in other omnivorous animals.

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APPENDIX 1: BREEDING AND WINTERING CATCHMENT AREAS OF YELLOW-RUMPED WARBLERS AT A MIGRATION MONITORING STATION

A1.1. INTRODUCTION

Knowledge of migratory connectivity between breeding, wintering, and stopover sites is important for a complete understanding of songbird ecology, evolution and making appropriate management decisions for conservation purposes (Webster et al. 2002). Events at one stage of the annual cycle of migratory songbirds influence events in subsequent stages at both individual and population levels.

Migration monitoring stations (MMS) were established to monitor changes in migratory populations of songbirds. Until recently, MMS were the only available option for monitoring population changes of species that may not be detected through other programs such as the Breeding Bird Survey (BBS) or Christmas Bird Count (CBC) (Dunn and Hussell 1995). The downside of this program is its inability to provide sufficient data to link breeding and wintering grounds mainly due to a low rate of band encounters for small passerines (Hobson 2003).

Stable hydrogen isotope analysis provides a new and complementary approach to studying connectivity. This technique works on the basis that the ratio of hydrogen to deuterium in precipitation varies systematically with latitude across North America and deuterium values in bird feathers can be traced back to the latitude where they were synthesized. Birds generally go through a complete body moult on or near the breeding grounds and a partial body moult on the wintering grounds (Pyle 1997) therefore by knowing the moult pattern of a species, stable isotope analysis of these specific feathers can provide breeding and wintering latitudes. Yellow-rumped Warblers have a pre-alternate moult prior to spring migration in which they replace greater coverts and a pre-basic moult post-breeding and prior to fall migration in which they replace all body and flight feathers. Thus, stable-hydrogen isotope analysis of greater coverts and tail feathers should provide latitudinal information on the wintering and breeding grounds, respectively. A number of studies published in recent years have demonstrated that stable hydrogen isotope analysis is a valid technique for delineating populations sampled at MMS in North America (Hobson et al. 1997; Wassenaar and Hobson 2001; Smith et al. 2003; Mazerolle et al. 2005).

The objective of this study was to delineate breeding and wintering catchment areas to determine where Yellow-rumped Warblers sampled by the Delta Marsh Bird Observatory (DMBO) are originating.

A1.2. METHODS

A1.2.1. Study Area

Field work was conducted at the DMBO, located in the dune-ridge forest of Delta Marsh, MB, Canada (98°23'W, 50°11'N), during spring (May 1 - 27, 2003) and fall (August 1 to September 30, 2003) migration of 2003. This forest abuts the south end of Lake Manitoba and is a primary stopover site for migratory songbirds *en route* to and from their breeding and wintering grounds. Large numbers of songbirds concentrate at the DMBO during migration, with an average of 7,500 birds caught per year (den Haan, unpublished data).

A1.2.2. Avian sampling

Yellow-rumped Warblers were captured using standard constant-effort mist netting and banding protocols recommended by Hussell and Ralph (1998). Feathers, two greater coverts and a tail feather (rectrix 4), were collected from each individual.

A1.2.3. Stable Isotope Analysis

Feathers were cleaned of surface oils with a 2:1 chloroform:methanol mixture and allowed to air dry. Stable hydrogen isotope analyses were performed at the National Hydrology Research Centre, Environment Canada, Saskatoon, SK. Approximately 33 mg of feather (0.31 – 0.37 mg) was weighed out and sealed into silver cups. The non-exchangeable hydrogen of feathers was measured using continuous-flow isotope-ratio mass spectrometer (CF-IRMS), as described by Wassenaar and Hobson (2002). Calibrated keratin isotope material was used a laboratory standard. Deuterium values are reported in δ (delta) notation, expressed as parts-per-thousand (‰) units, relative to the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale.

A1.2.4. Statistical Analysis

To estimate the breeding and wintering ground catchment areas of Yellow-rumped Warblers from the DMBO, 50 % and 75 % tolerance limits at 95 % confidence levels were calculated for δD values of greater coverts and tail feathers. δD values of feathers (δD_f) were converted to precipitation δD values (δD_p) by applying an isotopic discrimination factor of -25 ‰ (Wassenaar and Hobson 2001) in order to delineate the geographical catchment area. A geographic information system (GIS) based model of δD_p values (Bowen et al. 2005) was used to generate catchment areas. These areas were then constrained by the boundary of the geographic distribution of the species and by the 50 % and 75 % tolerance limits of δD values. Of the birds sampled at the DMBO, 50% and 75% of them originated from the areas located within the 50% and 75% tolerance intervals of δD values, respectively.

A1.3. RESULTS

Totals of 99 greater coverts and 82 tail feathers were sampled and analyzed for δD values for Yellow-rumped Warblers captured during spring and fall migration of 2003 (Table A1.1). Not surprisingly, mean δD values of greater coverts and tail feathers were significantly different (Fig. A1.1). Greater covert δD values corresponded to areas in the southeastern United States (Fig. A.1.2). Tail feather δD values corresponded to areas in the mid-boreal to high-boreal of western Canada and Alaska (Fig. A1.2).

A1.4. DISCUSSION

It was clearly demonstrated that the MMS at Delta Marsh sampled Yellow-rumped Warblers during fall migration from a broad catchment area across the Boreal forest using stable hydrogen isotope analyses of feathers. These findings concur with other studies that have used δD values of feathers to delineate breeding catchment areas from MMS (Hobson et al. 1997, Wassenaar and Hobson 2001, Smith et al. 2003, Mazerolle et al. 2005, Dunn et al. 2006). Furthermore, results of this study support the findings of Dunn et al. (2006) who also found that Yellow-rumped Warblers staging at the DMBO originated from mid-boreal to high-boreal breeding areas. As demonstrated by the δD values of greater coverts moulted on the wintering grounds, Yellow-rumped Warblers migrating through the DMBO during spring migration originated from a broad

Table A1.1 Descriptive statistics of δD values (‰) of greater coverts and tail feathers collected from Yellow-rumped Warblers staging at the Delta Marsh Bird Observatory, MB, during spring and fall migration of 2003, respectively.

	N	Mean	SD	SE
Greater coverts	99	-42.2	11.7	1.2
Tail feathers	82	-138.5	14.1	1.6

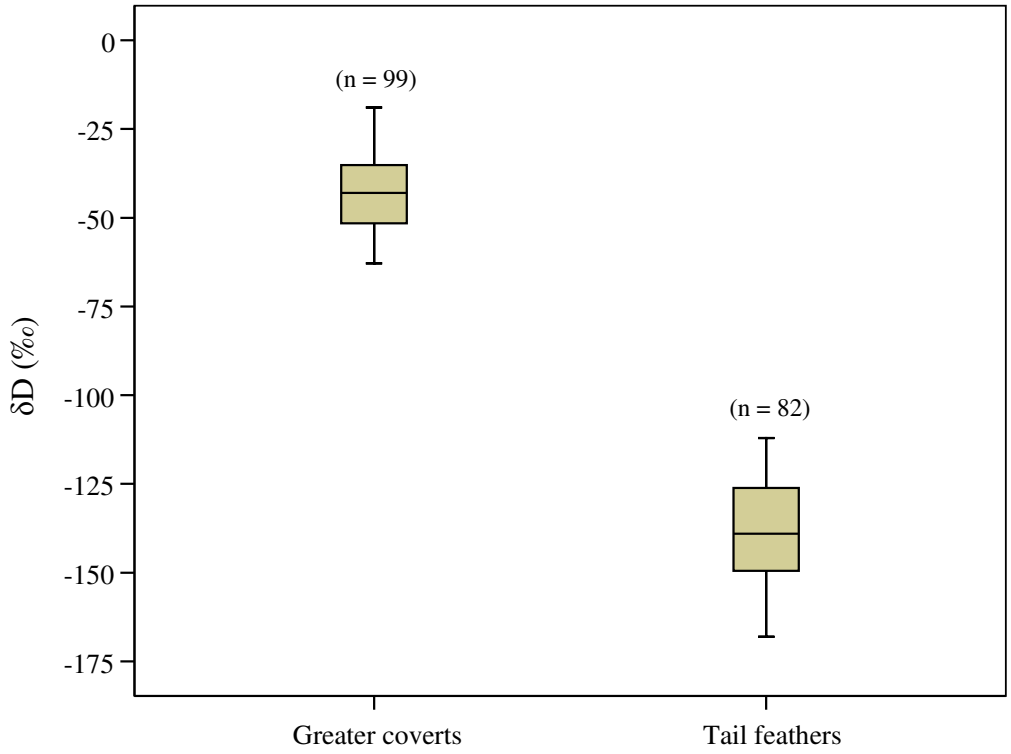

Figure A1.1 Box plots of the δD (‰) values of greater coverts and tail feathers from Yellow-rumped Warblers migrating through the Delta Marsh Bird Observatory, MB, during spring and fall migration 2003. Sample sizes are indicated in parentheses.

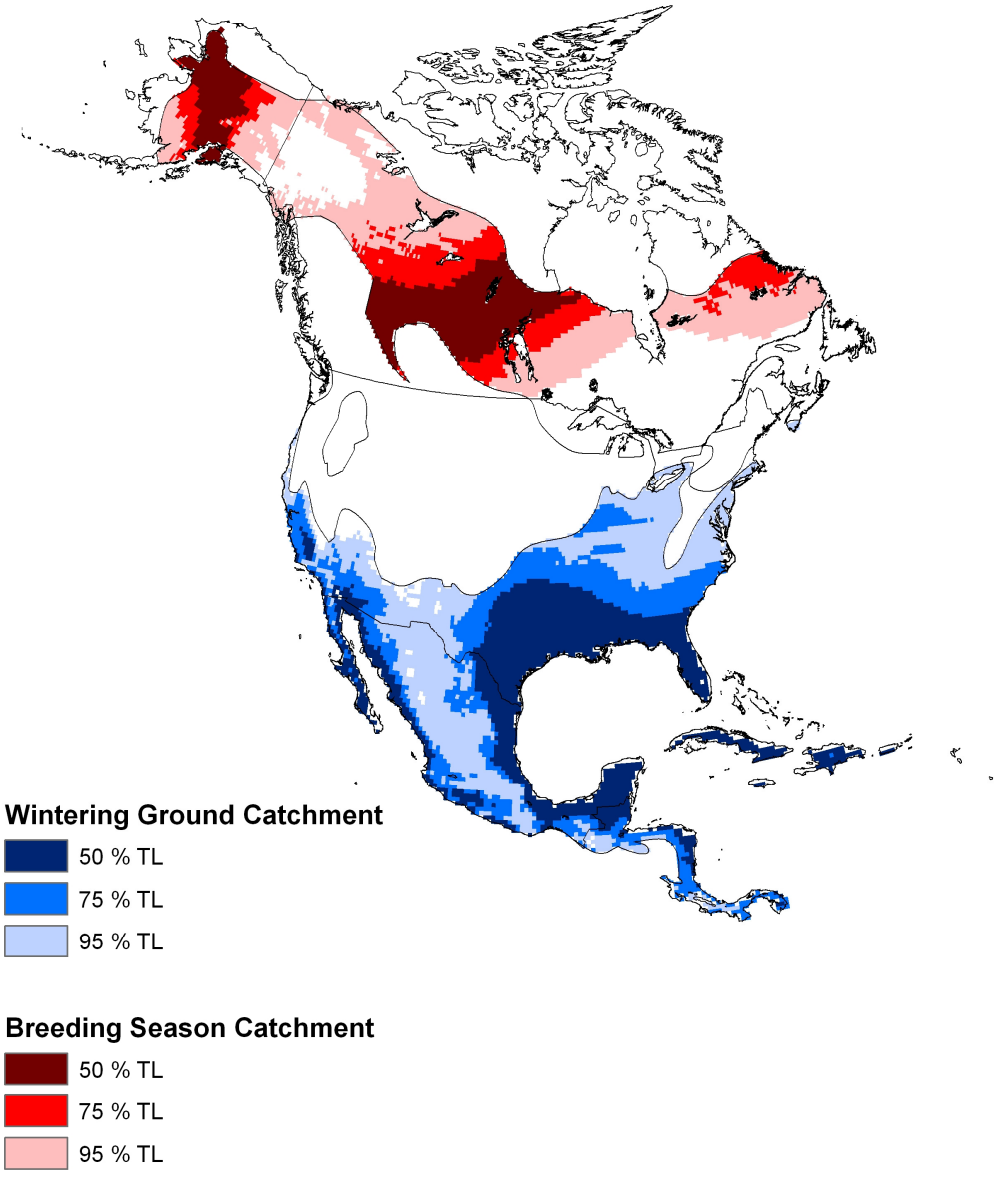

Figure A1.2 Estimated breeding and wintering catchment areas of Yellow-rumped Warblers captured at the Delta Marsh Bird Observatory, MB, during spring and fall migration periods in 2003. A GIS based model of feather δD values was used to generate the map. The catchment areas were constrained by the boundary of the geographic distribution of the species and by the 50 %, 75 % and 95 % tolerance limits of feather δD values. δD values of greater coverts (n = 99) and tail feathers (n = 82) were used to determine winter and breeding season catchment areas, respectively.

catchment area in the southeastern United States with a high proportion of individuals congregating along coastal areas during winter.

A1.5. LITERATURE CITED

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APPENDIX 2: SUPPLEMENTAL TABLES FROM CHAPTER 2

Table A2.1 Mean and 95% confidence intervals of blood $\delta^{15}N$ and $\delta^{13}C$ values for age classes of migratory songbirds staging at Delta Marsh, Manitoba, during spring migration of 2003. Only species with individuals representing both age classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. SY = second year, ASY = after second year.

					95% CI	
Species	Isotope	Age	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	SY	2	7.1	3.8	10.3
		ASY	4	5.8	3.3	8.2
	$\delta^{13}C$	SY	2	-23.5	-24.0	-22.9
		ASY	4	-23.3	-24.0	-22.7
BAOR	$\delta^{15}N$	SY	4	6.1	4.2	8.0
		ASY	2	6.5	2.0	11.0
	$\delta^{13}C$	SY	4	-23.8	-24.1	-23.4
		ASY	2	-23.4	-26.2	-20.5
CEDW	$\delta^{15}N$	SY	6	9.0	8.1	9.8
		ASY	3	7.8	5.4	10.3
	$\delta^{13}C$	SY	6	-24.1	-25.0	-23.2
		ASY	3	-24.7	-26.6	-22.8
GRCA	$\delta^{15}N$	SY	2	7.9	-10.4	26.3
		ASY	6	6.9	5.8	8.0
	$\delta^{13}C$	SY	2	-24.0	-24.5	-23.5
		ASY	6	-24.5	-25.4	-23.6
MAWA	$\delta^{15}N$	SY	3	5.8	2.2	9.4
		ASY	2	4.5	-4.3	13.2
	$\delta^{13}C$	SY	3	-24.3	-26.2	-22.5
		ASY	2	-23.4	-25.7	-21.1
YRWA	$\delta^{15}N$	SY	7	5.5	5.1	5.9
		ASY	3	4.7	3.2	6.2
	$\delta^{13}C$	SY	7	-23.6	-24.0	-23.2
		ASY	3	-24.6	-26.1	-23.2

Table A2.2 Mean and 95% confidence intervals of claw $\delta^{15}N$ and $\delta^{13}C$ values for age classes of migratory songbirds staging at Delta Marsh, Manitoba, during spring migration of 2003. Only species with individuals representing both age classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. SY = second year, ASY = after second year.

					95% CI	
Species	Isotope	Age	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	SY	4	6.7	4.6	8.7
		ASY	4	6.5	3.2	9.8
	$\delta^{13}C$	SY	4	-21.1	-23.4	-18.7
		ASY	4	-21.5	-22.4	-20.5
BAOR	$\delta^{15}N$	SY	4	7.0	4.6	9.4
		ASY	2	7.8	3.8	11.8
	$\delta^{13}C$	SY	4	-21.8	-23.1	-20.5
		ASY	2	-21.5	-22.6	-20.4
CEDW	$\delta^{15}N$	SY	8	6.4	5.3	7.5
		ASY	4	6.2	3.8	8.7
	$\delta^{13}C$	SY	8	-22.9	-23.4	-22.3
		ASY	4	-23.0	-25.1	-21.0
GRCA	$\delta^{15}N$	SY	2	7.1	-16.5	30.7
		ASY	6	6.0	4.4	7.6
	$\delta^{13}C$	SY	2	-22.9	-25.7	-20.1
		ASY	6	-23.2	-24.8	-21.7
MAWA	$\delta^{15}N$	SY	6	4.7	3.9	5.5
		ASY	3	4.2	3.7	4.8
	$\delta^{13}C$	SY	6	-21.8	-22.6	-21.1
		ASY	3	-22.3	-23.8	-20.7
YRWA	$\delta^{15}N$	SY	7	6.8	5.4	8.3
		ASY	3	5.7	3.3	8.0
	$\delta^{13}C$	SY	7	-21.8	-23.6	-19.9
		ASY	3	-22.2	-25.8	-18.6

Table A2.3 Mean and 95% confidence intervals of blood $\delta^{15}N$ and $\delta^{13}C$ values for age classes of migratory songbirds staging at Delta Marsh, Manitoba, during fall migration of 2003. Only species with individuals representing both age classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. HY = hatch year, AHY = after hatch year.

					95% CI	
Species	Isotope	Age	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	HY	5	6.0	4.4	7.6
		AHY	5	6.2	3.9	8.4
	$\delta^{13}C$	HY	5	-24.3	-25.7	-22.9
		AHY	5	-24.2	-24.7	-23.8
BAWW	$\delta^{15}N$	HY	6	5.1	4.0	6.3
		AHY	5	5.0	4.1	6.0
	$\delta^{13}C$	HY	6	-24.4	-24.7	-24.1
		AHY	5	-25.0	-25.9	-24.2
COYE	$\delta^{15}N$	HY	7	8.4	6.7	10.0
		AHY	3	8.7	6.6	10.8
	$\delta^{13}C$	HY	7	-24.9	-25.3	-24.4
		AHY	3	-24.7	-24.8	-24.6
HETH	$\delta^{15}N$	HY	5	6.9	6.1	7.7
		AHY	5	6.6	5.4	7.7
	$\delta^{13}C$	HY	5	-24.5	-25.4	-23.7
		AHY	5	-24.3	-24.7	-23.9
OCWA	$\delta^{15}N$	HY	8	4.6	4.1	5.1
		AHY	2	4.2	1.5	7.0
	$\delta^{13}C$	HY	8	-24.5	-25.	-24.1
		AHY	2	-24.5	-30.9	-18.2
SOSP	$\delta^{15}N$	HY	5	8.0	5.3	10.7
		AHY	5	8.9	7.0	10.8
	$\delta^{13}C$	HY	5	-24.1	-26.2	-22.1
		AHY	5	-25.2	-25.5	-24.8
YRWA	$\delta^{15}N$	HY	5	6.8	4.2	9.4
		AHY	5	3.9	3.5	4.3
	$\delta^{13}C$	HY	5	-24.4	-25.0	-23.8
		AHY	5	-24.3	-25.0	-23.7

Table A2.4 Mean and 95% confidence intervals of claw $\delta^{15}N$ and $\delta^{13}C$ values for age classes of migratory songbirds staging at Delta Marsh, Manitoba, during fall migration of 2003. Only species with individuals representing both age classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. HY = hatch year, AHY = after hatch year.

					95% CI	
Species	Isotope	Age	N	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	HY	5	6.0	4.6	7.4
		AHY	5	6.1	4.4	7.8
	$\delta^{13}C$	HY	5	-22.9	-24.0	-21.8
		AHY	5	-22.5	-23.2	-21.8
BAWW	$\delta^{15}N$	HY	6	4.6	3.4	5.8
		AHY	5	4.7	3.0	6.5
	$\delta^{13}C$	HY	6	-22.9	-23.5	-22.2
		AHY	5	-23.7	-25.1	-22.3
COYE	$\delta^{15}N$	HY	6	8.1	6.7	9.5
		AHY	3	8.8	7.2	10.4
	$\delta^{13}C$	HY	6	-23.4	-24.1	-22.6
		AHY	3	-23.0	-24.2	-21.9
HETH	$\delta^{15}N$	HY	5	5.6	4.6	6.6
		AHY	5	5.2	3.9	6.4
	$\delta^{13}C$	HY	5	-22.9	-23.6	-22.3
		AHY	5	-22.7	-22.9	-22.5
OCWA	$\delta^{15}N$	HY	8	3.8	3.2	4.4
		AHY	2	3.9	1.8	6.0
	$\delta^{13}C$	HY	8	-22.6	-22.8	-22.3
		AHY	2	-22.8	-23.7	-21.8
SOSP	$\delta^{15}N$	HY	5	7.5	5.5	9.5
		AHY	5	8.3	5.8	10.9
	$\delta^{13}C$	HY	5	-22.7	-24.3	-21.0
		AHY	5	-23.2	-23.5	-23.0
YRWA	$\delta^{15}N$	HY	5	5.2	3.0	7.3
		AHY	5	4.3	2.8	5.9
	$\delta^{13}C$	HY	5	-22.7	-23.3	-22.0
		AHY	5	-22.6	-23.2	-22.1

Table A2.5 Mean and 95% confidence intervals of blood $\delta^{15}N$ and $\delta^{13}C$ values for sex classes of migratory songbirds staging at Delta Marsh, Manitoba, during spring migration of 2003. Only species with individuals representing both sex classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. M = male, F = female.

					95% CI	
Species	Isotope	Sex	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	M	4	6.3	4.1	8.5
		F	2	6.0	-11.8	23.7
	$\delta^{13}C$	M	4	-23.4	-24.0	-22.7
		F	2	-23.4	-24.7	-22.1
BAOR	$\delta^{15}N$	M	5	6.1	4.8	7.4
		F	2	5.8	-7.5	19.1
	$\delta^{13}C$	M	5	-23.7	-24.0	-23.5
		F	2	-23.3	-25.5	-21.2
CEDW	$\delta^{15}N$	M	3	8.5	6.6	10.4
		F	6	8.6	7.4	9.9
	$\delta^{13}C$	M	3	-24.3	-27.5	-21.1
		F	6	-24.3	-25.0	-23.6
OCWA	$\delta^{15}N$	M	4	4.6	3.3	5.8
		F	6	5.8	4.4	7.2
	$\delta^{13}C$	M	4	-23.6	-23.8	-23.4
		F	6	-23.3	-25.3	-21.4
TRES	$\delta^{15}N$	M	4	9.5	8.9	10.2
		F	2	10.1	6.7	13.5
	$\delta^{13}C$	M	4	-23.0	-24.8	-21.2
		F	2	-22.5	-28.7	-16.3
YRWA	$\delta^{15}N$	M	10	5.2	4.5	5.8
		F	2	5.8	-4.7	16.2
	$\delta^{13}C$	M	10	-23.9	-24.4	-23.4
		F	2	-24.2	-33.8	-14.6

Table A2.6 Mean and 95% confidence intervals of claw $\delta^{15}N$ and $\delta^{13}C$ values for sex classes of migratory songbirds staging at Delta Marsh, Manitoba, during spring migration of 2003. Only species with individuals representing both sex classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. M = male, F = female.

					95% CI	
Species	Isotope	Sex	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	M	6	6.8	5.0	8.7
		F	2	5.9	-0.9	12.8
	$\delta^{13}C$	M	6	-21.0	-22.3	-19.8
		F	2	-22.0	-22.2	-21.8
BAOR	$\delta^{15}N$	M	5	7.1	5.5	8.8
		F	2	7.4	-2.5	17.2
	$\delta^{13}C$	M	5	-21.7	-22.6	-20.8
		F	2	-21.9	-27.4	-16.3
CEDW	$\delta^{15}N$	M	4	6.0	4.2	7.9
		F	8	6.5	5.3	7.7
	$\delta^{13}C$	M	4	-23.0	-24.8	-21.2
		F	8	-23.0	-23.6	-22.3
COYE	$\delta^{15}N$	M	11	7.1	5.7	8.4
		F	2	7.4	-12.4	27.2
	$\delta^{13}C$	M	11	-22.5	-23.1	-21.9
		F	2	-18.9	-45.7	7.8
OCWA	$\delta^{15}N$	M	4	4.3	2.2	6.4
		F	8	6.8	5.3	8.2
	$\delta^{13}C$	M	4	-22.6	-23.4	-21.9
		F	8	-21.9	-22.8	-20.9
TRES	$\delta^{15}N$	M	4	7.7	6.1	9.3
		F	2	7.8	4.3	11.4
	$\delta^{13}C$	M	4	-20.5	-22.4	-18.6
		F	2	-19.7	-29.2	-10.3
YRWA	$\delta^{15}N$	M	10	6.4	5.0	7.9
		F	2	5.0	-4.1	14.0
	$\delta^{13}C$	M	10	-21.8	-22.7	-21.0
		F	2	-21.1	-42.2	-0.01

Table A2.7 Mean and 95% confidence intervals of blood $\delta^{15}N$ and $\delta^{13}C$ values for sex classes of migratory songbirds staging at Delta Marsh, Manitoba, during fall migration of 2003. Only species with individuals representing both sex classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. M = male, F = female.

					95% CI	
Species	Isotope	Sex	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	M	5	6.1	3.8	8.4
		F	5	6.1	4.5	7.6
	$\delta^{13}C$	M	5	-24.1	-24.7	-23.4
		F	5	-24.5	-25.7	-23.2
BAWW	$\delta^{15}N$	M	6	5.4	4.4	6.4
		F	4	4.8	3.3	6.3
	$\delta^{13}C$	M	6	-24.7	-25.1	-24.2
		F	4	-24.7	-26.1	-23.4
OCWA	$\delta^{15}N$	M	3	4.1	2.6	5.6
		F	2	4.3	1.5	7.0
	$\delta^{13}C$	M	3	-24.2	-25.0	-23.4
		F	2	-24.5	-30.9	-18.2
YRWA	$\delta^{15}N$	M	3	5.0	0.8	9.3
		F	6	5.7	3.1	8.3
	$\delta^{13}C$	M	3	-24.3	-25.8	-22.9
		F	6	-24.4	-24.8	-23.9

Table A2.8 Mean and 95% confidence intervals of claw $\delta^{15}N$ and $\delta^{13}C$ values for sex classes of migratory songbirds staging at Delta Marsh, Manitoba, during fall migration of 2003. Only species with individuals representing both sex classes are included. See Table 2.1 for a species' full name corresponding with its 4-letter code. M = male, F = female.

					95% CI	
Species	Isotope	Sex	n	Mean (‰)	Lower	Upper
AMRE	$\delta^{15}N$	M	5	5.8	4.1	7.6
		F	5	6.2	4.9	7.6
	$\delta^{13}C$	M	5	-22.5	-23.3	-21.8
		F	5	-22.9	-24.0	-21.9
BAWW	$\delta^{15}N$	M	6	4.8	3.5	6.0
		F	4	4.8	2.4	7.1
	$\delta^{13}C$	M	6	-23.3	-24.2	-22.4
		F	4	-23.3	-25.3	-21.3
COYE	$\delta^{15}N$	M	7	8.3	7.1	9.5
		F	2	8.4	4.4	12.3
	$\delta^{13}C$	M	7	-23.3	-23.9	-22.6
		F	2	-22.9	-23.2	-22.5
OCWA	$\delta^{15}N$	M	3	3.6	2.4	4.8
		F	2	3.9	1.8	6.0
	$\delta^{13}C$	M	3	-22.4	-22.9	-21.9
		F	2	-22.8	-23.7	-21.8
YRWA	$\delta^{15}N$	M	3	4.7	2.4	7.1
		F	6	5.1	3.5	6.8
	$\delta^{13}C$	M	3	-22.6	-22.9	-22.4
		F	6	-22.6	-23.2	-22.0

Table A2.9 Results of linear regressions examining the influence of capture day (Julian date) on blood and claw $\delta^{15}N$ and $\delta^{13}C$ values sampled from songbirds captured at the Delta Marsh Bird Observatory, MB, during spring migration 2003. See Table 2.1 for a species' full name corresponding with its 4-letter code.

		Blood			Claws		
Species	Isotope	n	r^2	P	n	r^2	P
AMRE	$\delta^{15}N$	7	0.08	0.5	9	0.1	0.4
	$\delta^{13}C$	7	0.3	0.2	9	0.2	0.2
AMRO	$\delta^{15}N$	12	0.2	0.2	13	0.002	0.9
	$\delta^{13}C$	12	0.05	0.5	13	0.1	0.2
BAOR	$\delta^{15}N$	10	0.5	0.02	11	0.3	0.06
	$\delta^{13}C$	10	0.06	0.5	11	0.03	0.6
BAWW	$\delta^{15}N$	4	0.02	0.9	5	0.9	0.02
	$\delta^{13}C$	4	0.7	0.2	5	0.7	0.09
CEDW	$\delta^{15}N$	9	0.004	0.9	12	0.0	1.0
	$\delta^{13}C$	9	0.1	0.4	12	0.001	0.9
COYE	$\delta^{15}N$	12	0.4	0.02	17	0.2	0.1
	$\delta^{13}C$	12	0.006	0.8	17	0.1	0.1
GRCA	$\delta^{15}N$	10	0.05	0.5	10	0.2	0.2
	$\delta^{13}C$	10	0.001	0.9	10	0.005	0.8
HETH	$\delta^{15}N$	15	n/a	n/a	15	n/a	n/a
	$\delta^{13}C$	15	n/a	n/a	15	n/a	n/a
HOWR	$\delta^{15}N$	9	0.07	0.5	9	0.6	0.02
	$\delta^{13}C$	9	0.05	0.6	9	0.04	0.6
LEFL	$\delta^{15}N$	15	0.04	0.5	17	0.1	0.1
	$\delta^{13}C$	15	0.05	0.4	17	0.4	0.008
MAWA	$\delta^{15}N$	5	0.4	0.2	10	0.02	0.7
	$\delta^{13}C$	5	0.5	0.2	10	0.03	0.6
OCWA	$\delta^{15}N$	10	0.4	0.07	12	0.3	0.07
	$\delta^{13}C$	10	0.2	0.21	12	0.3	0.04
SOSP	$\delta^{15}N$	10	0.02	0.7	10	0.01	0.8
	$\delta^{13}C$	10	0.1	0.3	10	0.002	0.9
TRES	$\delta^{15}N$	10	0.08	0.427	9	0.003	0.9
	$\delta^{13}C$	10	0.4	0.040	9	0.2	0.2
WAVI	$\delta^{15}N$	10	0.02	0.7	10	0.3	0.1
	$\delta^{13}C$	10	0.2	0.2	10	0.004	0.9
YRWA	$\delta^{15}N$	15	0.0	1.0	15	0.2	0.07
	$\delta^{13}C$	15	0.005	0.8	15	0.2	0.06

n/a = all individuals were captured on the same day

Table A2.10 Results of linear regressions examining the influence of capture day on blood and claws $\delta^{15}N$ and $\delta^{13}C$ values sampled from songbirds captured at the Delta Marsh Bird Observatory, MB, during fall migration 2003. See Table 2.1 for a species' full name corresponding with its 4-letter code.

		Blood			Claws		
Species	Isotope	n	r^2	P	n	r^2	P
AMRE	$\delta^{15}N$	10	0.006	0.8	10	0.01	0.8
	$\delta^{13}C$	10	0.001	0.9	10	0.08	0.4
AMRO	$\delta^{15}N$	2	1.0	n/a	3	0.5	0.5
	$\delta^{13}C$	2	1.0	n/a	3	0.4	0.6
BAOR	$\delta^{15}N$	5	0.08	0.6	6	0.07	0.6
	$\delta^{13}C$	5	0.1	06	6	0.004	0.9
BAWW	$\delta^{15}N$	11	0.0	0.9	11	0.001	0.9
	$\delta^{13}C$	11	0.2	0.2	11	0.2	0.1
CEDW	$\delta^{15}N$	2	1.0	n/a	3	0.08	0.8
	$\delta^{13}C$	2	1.0	n/a	3	0.2	0.7
COYE	$\delta^{15}N$	10	0.07	0.4	10	0.04	0.6
	$\delta^{13}C$	10	0.1	0.3	10	0.1	0.4
GRCA	$\delta^{15}N$	8	0.7	0.01	10	0.6	0.005
	$\delta^{13}C$	8	0.1	0.4	10	0.003	0.9
HETH	$\delta^{15}N$	10	0.3	0.1	10	0.4	0.07
	$\delta^{13}C$	10	0.2	0.2	10	0.3	0.1
HOWR	$\delta^{15}N$	11	0.0	1.0	11	0.1	0.3
	$\delta^{13}C$	11	0.02	0.7	11	0.08	0.4
LEFL	$\delta^{15}N$	10	0.3	0.1	10	0.3	0.1
	$\delta^{13}C$	10	0.6	0.01	10	0.7	0.004
MAWA	$\delta^{15}N$	10	0.2	0.3	10	0.04	0.6
	$\delta^{13}C$	10	0.05	0.5	10	0.1	0.3
OCWA	$\delta^{15}N$	10	0.2	0.3	10	0.1	0.3
	$\delta^{13}C$	10	0.03	0.6	10	0.04	0.6
SOSP	$\delta^{15}N$	10	0.2	0.3	10	0.08	0.4
	$\delta^{13}C$	10	0.3	0.07	10	0.3	0.1
TRES	$\delta^{15}N$	11	n/a	n/a	11	n/a	n/a
	$\delta^{13}C$	11	n/a	n/a	11	n/a	n/a
WAVI	$\delta^{15}N$	10	0.03	0.7	10	0.03	0.6
	$\delta^{13}C$	10	0.4	0.07	10	0.04	0.6
YRWA	$\delta^{15}N$	10	0.6	0.006	10	0.4	0.07
	$\delta^{13}C$	10	0.01	0.8	10	0.006	0.8

n/a = all individuals were captured on the same day

Table A2.11 Results of linear regressions examining the influence of body condition on blood and claws $\delta^{15}N$ and $\delta^{13}C$ values sampled from songbirds captured at the Delta Marsh Bird Observatory, MB, during spring migration 2003. See Table 2.1 for a species' full name corresponding with its 4-letter code.

		Blood			Claws		
Species	Isotope	n	r^2	P	n	r^2	P
AMRE	$\delta^{15}N$	7	0.0	1.0	9	0.2	0.3
	$\delta^{13}C$	7	0.3	0.3	9	0.5	0.1
AMRO	$\delta^{15}N$	12	0.0	0.9	13	0.0	1.0
	$\delta^{13}C$	12	0.2	0.2	13	0.0	0.8
BAOR	$\delta^{15}N$	10	0.0	0.8	11	0.1	0.4
	$\delta^{13}C$	10	0.1	0.3	11	0.4	0.1
BAWW	$\delta^{15}N$	4	0.9	0.05	5	0.1	0.5
	$\delta^{13}C$	4	0.0	1.0	5	0.0	0.8
CEDW	$\delta^{15}N$	9	0.1	0.5	12	0.2	0.2
	$\delta^{13}C$	9	0.1	0.3	12	0.1	0.2
COYE	$\delta^{15}N$	12	0.3	0.1	17	0.1	0.1
	$\delta^{13}C$	12	0.0	0.8	17	0.0	0.4
GRCA	$\delta^{15}N$	10	0.0	0.6	10	0.1	0.4
	$\delta^{13}C$	10	0.2	0.1	10	0.5	0.03
HETH	$\delta^{15}N$	15	n/a	n/a	15	n/a	n/a
	$\delta^{13}C$	15	n/a	n/a	15	n/a	n/a
HOWR	$\delta^{15}N$	9	0.0	0.7	9	0.1	0.4
	$\delta^{13}C$	9	0.0	0.6	9	0.2	0.3
LEFL	$\delta^{15}N$	15	0.0	0.7	17	0.0	0.8
	$\delta^{13}C$	15	0.1	0.2	17	0.1	0.3
MAWA	$\delta^{15}N$	5	0.4	0.3	10	0.0	0.6
	$\delta^{13}C$	5	0.0	0.9	10	0.2	0.2
OCWA	$\delta^{15}N$	10	0.1	0.4	12	0.0	0.8
	$\delta^{13}C$	10	0.0	0.7	12	0.3	0.1
SOSP	$\delta^{15}N$	10	0.1	0.4	10	0.1	0.6
	$\delta^{13}C$	10	0.0	0.8	10	0.0	0.9
TRES	$\delta^{15}N$	10	0.0	0.7	9	0.0	0.7
	$\delta^{13}C$	10	0.2	0.2	9	0.0	0.7
WAVI	$\delta^{15}N$	10	0.1	0.3	10	0.0	0.8
	$\delta^{13}C$	10	0.0	0.8	10	0.3	0.1
YRWA	$\delta^{15}N$	15	0.1	0.3	15	0.1	0.4
	$\delta^{13}C$	15	0.1	0.3	15	0.1	0.4

n/a = all individuals were captured on the same day

Table A2.12 Results of linear regressions examining the influence of body condition on blood and claws $\delta^{15}N$ and $\delta^{13}C$ values sampled from songbirds captured at the Delta Marsh Bird Observatory, MB, during fall migration 2003. See Table 2.1 for a species' full name corresponding with its 4-letter code.

		Blood			Claws		
Species	Isotope	n	r^2	P	n	r^2	P
AMRE	$\delta^{15}N$	10	0.6	0.02	10	0.6	0.01
	$\delta^{13}C$	10	0.5	0.04	10	0.6	0.02
AMRO	$\delta^{15}N$	2	n/a	n/a	3	n/a	n/a
	$\delta^{13}C$	2	n/a	n/a	3	n/a	n/a
BAOR	$\delta^{15}N$	5	0.9	0.02	6	0.0	0.8
	$\delta^{13}C$	5	0.3	0.3	6	0.8	0.01
BAWW	$\delta^{15}N$	11	0.0	0.7	11	0.0	0.8
	$\delta^{13}C$	11	0.2	0.2	11	0.0	0.8
CEDW	$\delta^{15}N$	2	n/a	n/a	3	n/a	n/a
	$\delta^{13}C$	2	n/a	n/a	3	n/a	n/a
COYE	$\delta^{15}N$	10	0.1	0.5	10	0.1	0.3
	$\delta^{13}C$	10	0.0	0.9	10	0.1	0.5
GRCA	$\delta^{15}N$	8	0.1	0.4	10	0.1	0.5
	$\delta^{13}C$	8	0.1	0.5	10	0.0	0.7
HETH	$\delta^{15}N$	10	0.1	0.4	10	0.0	1.0
	$\delta^{13}C$	10	0.0	0.7	10	0.2	0.2
HOWR	$\delta^{15}N$	11	0.1	0.3	11	0.0	0.9
	$\delta^{13}C$	11	0.3	0.1	11	0.3	0.1
LEFL	$\delta^{15}N$	10	0.0	0.7	10	0.0	1.0
	$\delta^{13}C$	10	0.2	0.2	10	0.0	0.6
MAWA	$\delta^{15}N$	10	0.0	0.9	10	0.0	0.7
	$\delta^{13}C$	10	0.1	0.3	10	0.2	0.3
OCWA	$\delta^{15}N$	10	0.0	0.8	10	0.2	0.2
	$\delta^{13}C$	10	0.3	0.1	10	0.2	0.1
SOSP	$\delta^{15}N$	10	0.1	0.5	10	0.1	0.5
	$\delta^{13}C$	10	0.1	0.4	10	0.1	0.4
TRES	$\delta^{15}N$	11	n/a	n/a	11	n/a	n/a
	$\delta^{13}C$	11	n/a	n/a	11	n/a	n/a
WAVI	$\delta^{15}N$	10	0.0	0.9	10	0.0	0.9
	$\delta^{13}C$	10	0.0	0.7	10	0.0	0.6
YRWA	$\delta^{15}N$	10	0.3	0.1	10	0.1	0.4
	$\delta^{13}C$	10	0.0	0.9	10	0.1	0.3

n/a = all individuals were captured on the same day